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FAMILY MERIT AND INDIVIDUAL MERIT AS BASES FOR SELECTION.[1] PART I

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"FAMILY" is a word often used in plant and animal breeding, although with variable meaning. Always it conveys the idea that members of the same family are more like each other than they are like members of other families. Yet rarely does this go so far as to imply that members of the same family are *exactly* alike, or that two families do not overlap at all. The primary basis of family is always relationship by descent, with any outward or phenotypic similarity being a result of that. When some useful end or practical convenience is served by classifying individuals primarily according to their own phenotypes, the resulting groups are called by some such term as "types" or "classes" or "kinds" and are not called families.

The present study began with the practical problem of deciding how much attention ought to be paid to the merits and defects of litter mates when choosing boars and gilts to use for breeding. In making choices based on more than one criterion it is necessary to have some score, index or other systematic way of weighting and pooling all the relevant facts concerning each pig. Otherwise the actual choices will be swayed too much or too little by different items at different times.

A plausible formula for giving a pig "sib credits"

[1] Journal paper No. J–1439 of the Iowa Agricultural Experiment Station, Project No. 32.

according to the average merit of its litter mates was readily derived. After several years of practice in using these sib credits when selecting pigs, a more complete inquiry into the principles and possibilities of family selection was made as part of some studies made while on leave at the Kimber Poultry Breeding Farm, Niles, California.[2] The present paper presents the principles and formulas derived from these studies and from experience in using sib credits.

Statement of the Problem

The question to be considered here is how much the population mean would be changed in one generation by selecting on each of three different bases. Those three are: (a) The individual's own phenotypic merit (*i.e.*, mass selection); (b) the average merit of the family, wherein the entire family is selected or rejected (family selection); and (c) the optimum combination of some attention to individual merit plus some attention to the average merit of the family.

The differences between the three methods can be made clearer by the example in Fig. 1 which shows the 180-day weights of four pigs in each of four litters. Suppose that four of the pigs are needed for breeding and that heavier weights are desired. If selection is wholly on a family basis the selected pigs will be all four of those in litter 2, since that family has the highest average. Pig E, in spite of its very low weight, will be selected along with the other three. If selection is wholly on an individual basis, pigs *D*, *G*, *H* and *L* will be selected, regardless of the merits of their sibs. If the method of selection is some compromise, based partly on individual phenotype and partly on family average, pigs *F* and *P* might be saved instead of *D* and *L*.

Two things about this example deserve emphasis. First, pigs *G* and *H* will be saved, no matter which method

[2] John E. Kimber's interest in and support of this research is gratefully acknowledged.

of selection is used. The family average can be high only when more than a fair share of the individuals in that family are above average. Mass selection results in choosing some of the same individuals as would be chosen under purely family selection. If either method *is absolutely ineffective the other will be also.* Second, one can not pay attention both to family and to individuality without compromising on both. It will almost never happen that *all* members of the family which has the highest average will be individually superior to *all* members of the other families. The difference between the three

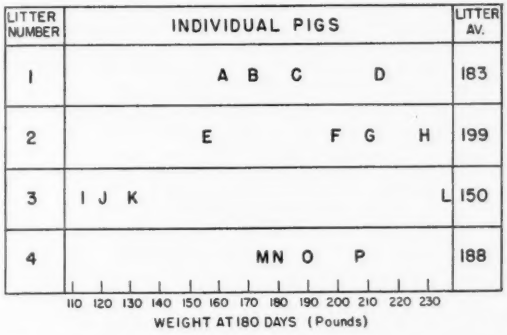


FIG. 1. Example to show what is meant by individual selection, by family selection, and by combination selection.

methods consists in what is to be done with good individuals (like D and P) from mediocre families, and with medium individuals (like F) or poor individuals (like E) from good families.

Regardless of why the breeder thinks he made his selections, the results of his having made them depend only on the extent to which the genetic composition of those selected to be parents differs from the genetic composition of those which would have been parents if no selection, or if some other method of selection, had been practiced. The process of selection really consists only of predicting the breeding value of each individual which is being considered and then keeping it or culling it on

the basis of that prediction. If the same fraction of the population must be saved but there is a choice of bases on which selection may be made, then the difference in results depends only on how accurately each individual's breeding value can be predicted from each of these bases.

Definitions and Notation

For comparing the effectiveness of the methods, the only biometrical descriptions of the family needed are the correlation (t) between phenotypes of members of a family, and the correlation (r) between breeding values of members of a family.

The numerical value of t for a particular characteristic can be measured directly for each population of families, although the fiducial limits of the figure thus obtained will be wide if the number of individuals in the sample on which it was measured is small.

The numerical value of r is given closely enough for practical purposes by Wright's coefficient of relationship if all the families belong to one breed, race or other population which was more or less freely interbreeding at a date only a few generations back, and if the relationship is computed to that date as a base. This is the usual practical case in which selection is practiced. For more complicated cases, such as selecting within a heterogeneous population containing different races, breeds or other groups whose ancestry has been separate for many generations, r is more difficult to measure. In such cases r is likely to have been augmented by the irregular incidence of mutations and by the accidents of sampling inherent in Mendelian inheritance. It may also have been either augmented or decreased by selection having been in different directions in the various groups or steadily in the same direction in them all. The definition of r, however, remains the same: the intraclass correlation between breeding values of members of the same family.

The following will illustrate kinds of families described by different values of r when all of r is due to relation-

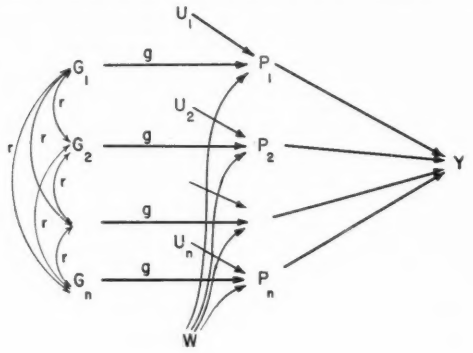
ship by descent: (1) If each family consists of a set of full sibs in a population in which the different sets of full sibs are unrelated to each other, r is .5. No higher value of r can be had in sexually reproducing organisms unless some inbreeding is practiced. Family is widely used in this sense when speaking of swine, of poultry and concerning human affairs. (2) If by family is meant the progeny of a single self-fertilized individual which was not itself produced by inbreeding, then r is 2/3. This is a common usage of family in plant breeding (*e.g.*, Mendel's F_3 families of peas), but is unknown in animal breeding since self-fertilization is not possible there. (3) If each family is a set of full sibs in a population of unrelated lines already inbred full brother and sister for two generations, r is 8/11. This condition is approached rather soon in animals when intensely inbred and unrelated lines are being established. (4) If each family is a set of half sibs in a population in which the different sets of half sibs are unrelated, r is .25. With the less prolific farm animals, such as cattle and sheep, it is difficult to produce large families with r much higher than this unless some inbreeding is done. (5) If a family is defined as all the grandsons and granddaughters of an individual and if the members of a family are unrelated to each other except through the one common grandparent, then r is .0625. This comes close to a rather frequent usage in animal husbandry when reference is made to "families in the male line" or to "families in the female line."

Biometrical Principles

Fig. 2 shows, in terms of path coefficients, the biometrical relations between the average phenotype (Y) of a family and the breeding values (G) and phenotypes (P) of the individual members of that family. W indicates all circumstances or conditions, other than the G's, which affect P in the same way for all members of a family but may differ from one family to another. U_1, U_2 - - - - - U_n

indicate all circumstances, except the G's, which affect the corresponding P's but are no more alike for members of the same family than they are for individuals which belong to different families.

An individual's "breeding value" is the sum of the average effects of the genes which it contains. This is what Fisher (1930, page 32) calls the "expected value."



$$r_{GP} = g$$

$$r_{GY} = g\frac{1+(n-1)r}{\sqrt{n[1+(n-1)t]}}$$

$$r_{PY} = \sqrt{\frac{1+(n-1)t}{n}}$$

$$t = r_{PP} = g^2r + r^2_{WP}$$

FIG. 2. Path coefficient diagram of biometrical relations involved in mass, family, and combination selection.

If the actual effects of a gene substitution upon P are sometimes larger and sometimes smaller than the average effect of that gene substitution in that population, depending upon what other genes are present, the differences between the actual and average effects are termed epistatic or dominance deviations. These give rise to "special" breeding values. That is, they cause some matings to produce better offspring and some to produce poorer ones than would be expected if one knew

the kind of offspring those same parents would produce if mated to a representative sample of the whole population. Most of the dominance and epistatic deviations from the additive scheme are included in U, but a small part are in W.

These biometrical relations may be clearer if stated also in terms of the variation of P. Consider that the individual's phenotype is completely determined by G, U and W. Then the variation in phenotypes can be described in terms of differences among the G's, U's and W's as follows:

A = the variance of P caused by differences among the G's,
E = the variance of P caused by differences among the U's, and
C = the variance of P caused by differenees between the various W's in the whole population, whence
A + E + C = the phenotypic variance between individuals belonging to different families.

Then t is the phenotypic counterpart of r, since,

$$r = \frac{\text{genetic variance between means of families of infinite size}}{\text{genetic variance between individuals from different families}} \text{ and}$$

$$t = \frac{\text{phenotypic variance between means of families of infinite size}}{\text{phenotypic variance between individuals from different families}}.$$

In terms of variance given above, $t = \frac{rA + C}{A + C + E}$. Thus t will be the same as r only when C and E are zero, as for a perfectly hereditary characteristic without dominance or epistasis, or when $\frac{C}{C+E} = r$. When E is large, t will be small. When C is large, t will be large and may even exceed r, especially if the latter is small.

Estimating Breeding Value

The change which a generation of selection based solely on criterion X will produce in the mean G of the next generation (and hence also in the mean P of an unselected sample of that generation) is expressed in the usual regression equation:

$\hat{G} = \text{Mean } G + r_{GX} \cdot \frac{\sigma G}{\sigma X} \cdot \frac{z}{b} \cdot \sigma X$ where b is the fraction saved for breeding, and z is the ordinate of the normal curve[3] at the level of X above which all are saved and below which all are culled. The expected increase, ΔG, in the population mean as a result of one generation of such selection is therefore simply $r_{GX} \cdot \sigma G \cdot \frac{z}{b}$.

[3] The slight qualifications necessary for z and b when the population is not distributed normally are discussed later.

For comparing in the same population the three methods of selection which are the subject of the present paper, σG and $\frac{z}{b}$ are constants. Hence progress (ΔP) under the three methods is directly proportional to r_{GP}, r_{GY} and r_{GI}, where I is the index or most probable breeding value of an individual, as estimated from the optimum linear combination of attention to its own phenotype and attention to the average phenotype of the family to which it belongs.

Reduced to terms of r, t, n, and the three parts of the variance of P, the expected increase in the population mean as a result of one generation of selection becomes:

(a) When selection is purely on individual merit (P):

$$\frac{z}{b} \cdot \frac{A}{\sqrt{A+C+E}} \tag{1}$$

(b) When selection is solely on the family average (Y):

$$\frac{z}{b} \cdot \frac{A}{\sqrt{A+C+E}} \cdot \frac{1+(n-1)r}{\sqrt{n[1+(n-1)t]}} \tag{2}$$

(c) When selection is on I, the best linear combination of P and Y:

$$\frac{z}{b} \cdot \frac{A}{\sqrt{A+C+E}} \cdot \sqrt{1+\frac{(r-t)^2}{1-t}\,\frac{n-1}{1+(n-1)t}} \tag{3}$$

The term, $\frac{A}{\sqrt{A+C+E}}$, is very important as related to the amount of improvement accomplished in absolute units. However, since it is common to all three methods, their *relative* effectiveness can be stated entirely in terms of r, t and n.

Mass Selection Compared with Family Selection

Purely family selection thus produces $\frac{1+(n-1)r}{\sqrt{n[1+(n-1)t]}}$ times as rapid progress as mass selection. This ratio exceeds 1.0 when $t < r^2 - \frac{(1-r)^2}{n}$. Hence purely family selection will produce less progress than mass selection whenever t is as large as r^2. Fig. 3 shows for several values of n the combinations of r and t at which family selection and mass selection are equally effective. Com-

binations of r and t, which lie to the right of these lines, are the situations in which purely family selection makes more rapid progress than mass selection. This is the region in which r has high values and t has low values. To the left of those lines, where r is low and t is high, is the region in which individual selection is more effective than family selection.

Fig. 3 makes the following conclusions clear: First,

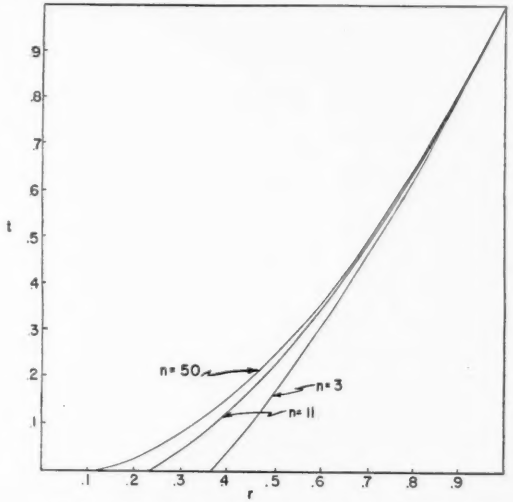


FIG. 3. To the left of the lines mass selection produces larger results than family selection; to the right are the combinations of r and t which make family selection more effective than mass selection.

making n large improves the effectiveness of family selection considerably only when t is very small. Second, for family selection to be more effective than individual selection, family members must resemble each other genetically much more than they do phenotypically, *i.e.*, r must be much larger than t. As long as inbreeding is avoided r will be .5 or less and family selection will be importantly superior to individual selection only for characteristics for which t is extremely small. Third, paradoxical though it may seem, family selection

is most superior to mass selection *when family members resemble each other least; i.e.,* when the families overlap widely in their phenotypes and t is therefore low! This is a far cry from the naive view, repeatedly disproven but still often inferred to be axiomatic, that family, breed and race are unimportant, or even unreal, unless the families, breeds or races are so distinct that they do not overlap at all.[4]

Selection on a Combination of Individual and Family Merit

The superiority of combination selection over mass selection depends mostly on the size of r–t, although n and the absolute size of t do have some influence. Fig. 4 gives a rough idea of the domain, among all possible values of r and t, in which paying attention also to family merit will make progress distinctly more rapid than could be made by mass selection alone. The primary fact which emerges from Fig. 4 is that r and t must be very unequal if combination selection is to make much more progress than would be made by mass selection alone. In the lower right-hand corner, where r is far larger than t, adding family selection properly to mass selection will increase progress very much. In the upper left-hand corner, where t is far larger than r, considering family merit also will increase considerably the effectiveness of the selection. But here the attention to family merit should be negative, as will be discussed in detail later.

Fig. 5 compares the effectiveness of combination and mass selection when n is 5. Each curve shows primarily the effects of changes in t, other conditions remaining constant. The five curves shown are for the values r would have in families of half sibs, "three-quarter" sibs,

[4] Many others might be cited, but a shining example is Jacques Barzun's "Race, a Study in Modern Superstition" (Harcourt, Brace and Company, 1937). See also Herskovits's reproof (*Science,* 101: 200) of students who ". . . make a case for racial differences where *the distinction is one of degree rather than kind,* . . ." (italics mine).

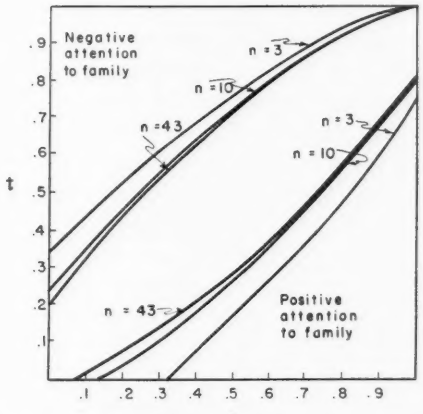


FIG. 4. The center is the region in which combination selection is less than 1.1 times as effective as mass selection.

full sibs, full sibs inbred brother and sister for one generation and full sibs inbred brother and sister for two generations. If n were larger the curves would be much higher near the extreme left, where t is much smaller than r, but the heights of the curves everywhere else would be altered very little and the points of tangency to

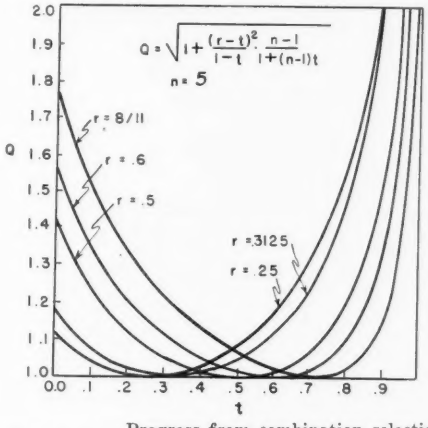


FIG. 5. Showing how $Q = \frac{\text{Progress from combination selection}}{\text{Progress from mass selection}}$ varies with r and t when $n = 5$.

the bottom line would remain exactly the same. Fig. 5 emphasizes that little is gained by paying attention also to family unless r and t are very unequal. When t is low, the gain from paying attention also to family merit rises markedly with r. When t is high, the gain from paying attention also to family merit,—negative attention to it in this case,—is highest when r is lowest.

Figs. 6, 7 and 8 show the comparative effectiveness of the three methods of selection, especially as affected by variations in n. The height of the horizontal line indi-

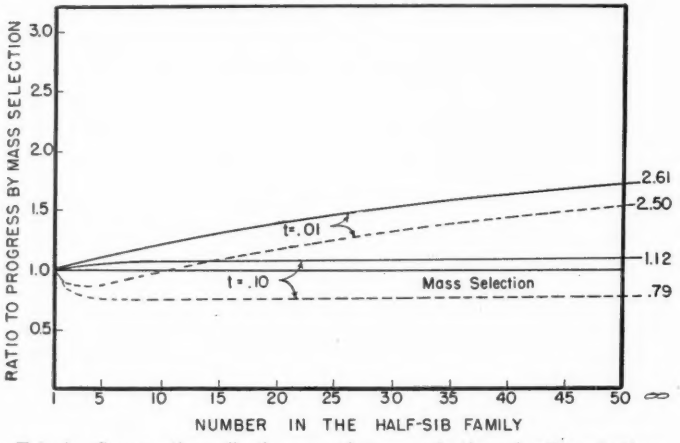


FIG. 6. Comparative effectiveness of mass selection, family selection, and combination selection when $r = .25$, as between half sibs.

cates the progress which would be made by mass selection alone. The broken lines show for comparison how much improvement would result from purely family selection. The curved solid lines show how much progress would be made by combination selection.

Figs. 6, 7 and 8 are drawn for cases where r is equal to .25, .50 and 2/3, respectively. The general conclusion to be drawn from these three figures is that when t is very small family selection and combination selection are nearly equal, their advantage over mass selection increasing distinctly with larger family size. At inter-

mediate levels of t combination selection is distinctly better than either of the others but less is gained by making n large. Finally, when t comes near to equalling r, combination selection is only a little better than mass selection and purely family selection is actually worse.

The differences between Figs. 6, 7 and 8 show that there is a real reason for plant breeders having generally put more faith in and made more use of family selection than most animal breeders have done. Fig. 6 especially

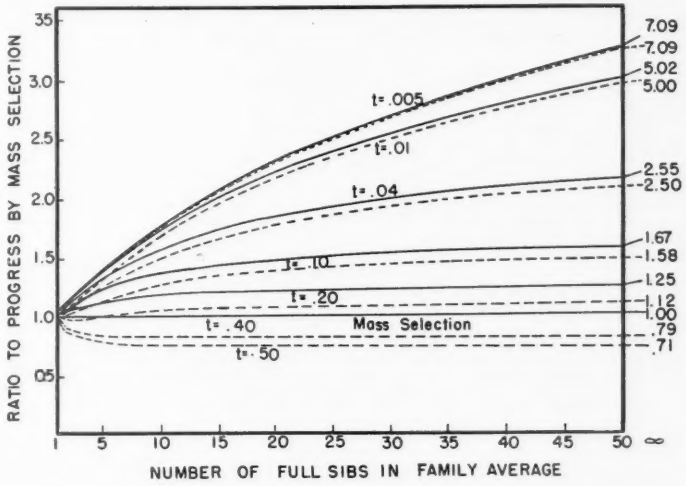


FIG. 7. Effectiveness of the three kinds of selection when $r = .5$, as when each family is a set of full sibs.

may make it plain that breeders of animals less prolific than poultry and swine are perhaps not grossly negligent after all if they actually give only a little lip service to the idea of family selection! With such animals it is difficult to get families with r much above .25 without inbreeding. Even with swine and poultry, where families with r of .5 are frequent, the family size is often less than it would be with most plants. Those plant breeders who work with material in which selfing is not feasible and clones do not occur will naturally find their use of family selection rather like that pictured in Fig. 7.

Figs. 9 and 10 show in another way the effectiveness of the three methods at all combinations of r and t. In both figures the progress which would result from one generation of mass selection is pictured as a level plane at height 1.0 above the base. That plane, where not hidden by the surface above it, is indicated by closely ruled diagonal lines. The curved upper surface, marked by heavy lines at each interval of .10 in r, shows the

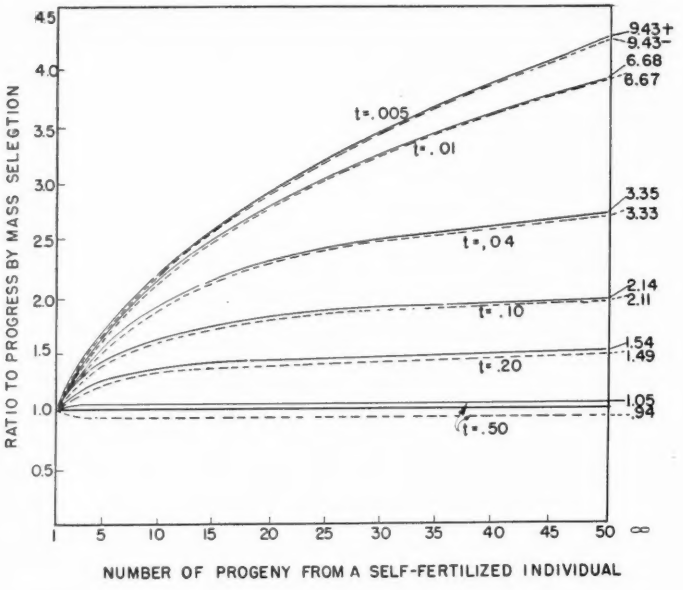


FIG. 8. Effectiveness of the three kinds of selection when $r = 2/3$, as when each family is the progeny of a self-fertilized F_2 plant.

progress which would result from combination selection. The dotted surface, visible only at the lower edges, shows what would result from purely family selection. The surface for family selection is tangent to the surface for combination selection at the left upper edge where $r = 1.0$, but for a constant value of t it slopes downward linearly with r toward the edge visible at the lower right where $r =$ zero.

In the center, along the diagonal connecting the two corners where r and t are both 1.0 or are both zero, is the valley in which combination selection is exactly equal to mass selection. Toward the left corner, where r is large and t is small, is the region where family selection is distinctly superior to mass selection and comes close to equalling combination selection. Toward the corner at the right the family average is extremely ineffective

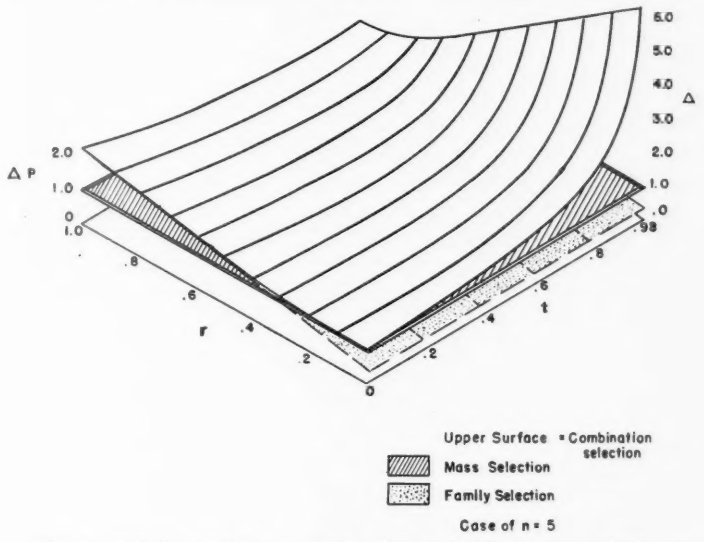


FIG. 9. Relative effectiveness of the three methods at all combinations of r and t when families consist of only five individuals.

alone but, when used negatively in the combination, it makes combination selection much more effective than mass selection. The apparently tremendous superiority of combination selection in the corner at the right may be a bit misleading, since this is drawn relative to the effectiveness of mass selection, which in this corner is low. For t to be close to 1.0 while r was low would mean that nearly all the individual phenotypic differences were caused by environmental things (W in Fig. 2) which were alike for all members of a family but differed from family

to family. In such a situation none of the three methods would produce rapid progress in absolute units, but the combination is much the most effective among the three and purely family selection is much the least.

Figs. 9 and 10 are nearly alike except in the left corner where t is low and r is high. Here large family size makes both family selection and combination selection much more superior to mass selection than they are when

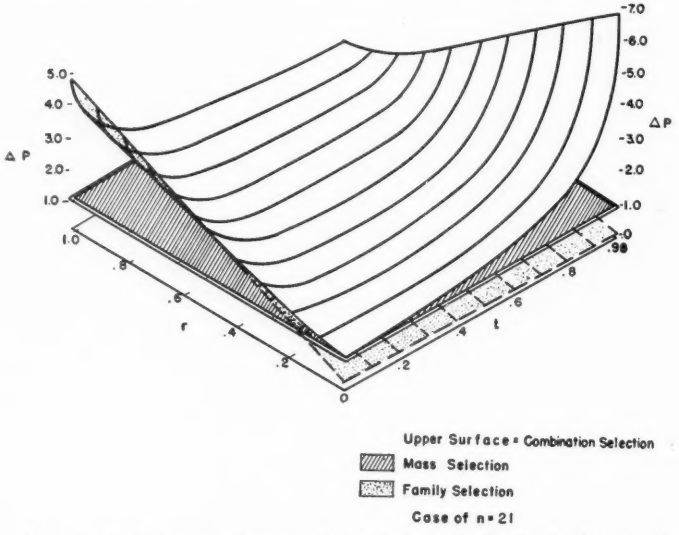


FIG. 10. Relative effectiveness of the three methods of selection at all combinations of r and t when families contain 21 individuals.

the families are small. This is another way of showing what was shown in Figs. 7 and 8 also; *i.e.*, family size does not make much difference except when t is small and r is large.

RELATIVE ATTENTION TO INDIVIDUAL MERIT AND TO FAMILY MERIT

In the preceding section it was shown that selection on the most efficient linear estimate of individual performance and family average was equal or superior to

mass selection and also to family selection for all values of r, t and n. The equation for predicting individual breeding value from the individual's own phenotype (P) and its family average (Y) can be written:

$$\hat{G} - \bar{G} = \frac{A}{A+C+E}\left\{\frac{1-r}{1-t}(P-\bar{P}) + \frac{r-t}{1-t}\cdot\frac{n}{1+(n-1)t}(Y-\bar{P})\right\}. \quad (4)$$

The net regression on individual phenotype, $\frac{A}{A+C+E}\cdot\frac{1-r}{1-t}$, is $\frac{\text{individual genetic variance within families}}{\text{individual phenotypic variance within families}}$, or the heritability of intra-family phenotypic differences. Where family size varies, it is convenient to construct a table giving correct values for the regression on family average and to use this in actual operations.

When the individual is the only one in the family ($n=1$,) its phenotype and its family average are identical and the sum of the two regression coefficients is $\frac{A}{A+C+E}$, which is simply the regression of breeding value on phenotype under mass selection. Preliminary selections can be made on this basis when it is expedient to cull some of the very worst individuals as soon as their own merit is known, without waiting until family averages are complete. Unless r and t are very different, one can thus discard early many of the very poorest individuals without much risk of discarding some which, when the family averages are complete, he will wish he had kept.

Fig. 11 shows the ratio between the net regressions on family average and on individual phenotype for the case $n=21$. Curves for this ratio, $\frac{r-t}{1-t}\cdot\frac{21}{1+20t}$, are shown for several values of r. This ratio is zero when $r=t$ and slightly negative when r is less than t. But when r far exceeds t the regression on family average can be many times that on individual phenotype. This situation is approached when selecting within a population of inbred but unrelated lines for characteristics which are

low in heritability. In such a case the family average is nearly all-important and the individual's own phenotype contains only a little additional information about its breeding value.

The term, $\frac{n}{1+(n-1)t}$, is the ratio of variance among individual phenotypes to the variance among actual family averages. This automatically adjusts the regression on family averages so that a difference between individuals and an equal difference between family averages will always be given importance inversely proportional to the variance of each. Changes in n affect the

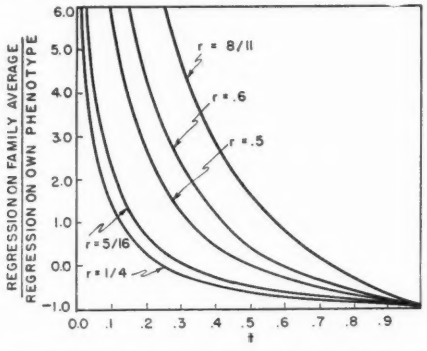


FIG. 11. Regression on family average as compared with regression on the individual itself when $n = 21$.

regression on the family average only slightly unless t is very small. Increasing n from one to five will not double the regression on family average unless t is less than .38 and will not triple it unless t is less than .17. Increasing n from five toward infinity will not double the regression on family average unless t is less than .17 nor triple it unless t is less than .10. Unless t is very small, most of the advantage of having n large is already achieved by the time n is large as four or five.

The meaning of the regression coefficients becomes still clearer (although they are less convenient for use) if the individual is considered as a deviation from its family average, instead of a deviation from the general popu-

lation. This can be done simply by writing $(P - Y + Y - \overline{P})$ in place of $(P - \overline{P})$ in equation (4) and then separating the first two terms from the last two. Then the equation for predicting the general breeding value (G) of an individual becomes:

$$\hat{G} - \overline{G} = \frac{A}{A + C + E} \left\{ \frac{1-r}{1-t} \cdot (P-Y) + \frac{1 + (n-1)r}{1 + (n-1)t} \cdot (Y - \overline{P}) \right\}. \qquad (5)$$

The first regression coefficient remains the same as before in equation (4). The second regression coefficient is now simply:

$$\frac{\text{Additively genetic variance between family averages}}{\text{Phenotypic variance between family averages}}$$

This is the heritability of observed differences between family averages.

In this form the regression coefficient for the family average can not be negative, although it can be very small if t exceeds r and if A is only a small part of the individual phenotypic variance. As n becomes large this regression on family average moves from unity toward r/t times the heritability of individual differences in the whole population. The fraction r/t can become extremely large in a population of inbred but unrelated families whose means differ only a little but within each of which there is much environmental variation.

Panse (1940, pp. 285–287) has published similar equations for estimating the breeding value of individual plants grown in different plots, using the plot average as the family average is used here. However, in his problem the plants in each plot were a random sample of the whole population of plants, *i.e.*, r was zero and all of t was environmental. The numerators of his regression coefficients simplified accordingly.

Negative Attention to Family

That the regression of individual breeding values on family average (when expressed as in equation 4) turns negative when t exceeds r may seem strange at first. However, when family members resemble each other phenotypically more than they do genetically, some fam-

ily averages must have been shoved far up and some far down by things other than the average breeding value of the family. When this happens, the family average will tell more about important environmental conditions which were peculiar to the family than about the breeding values of the individual members.

The family average contains two different kinds of information. First, it gives some indications of the average breeding value of the family. This is the rA part of t. Second, it indicates the special environmental conditions to which the whole family was exposed. This is the C part of t. Information of the first kind is useful positively, since the individual's own breeding value is likely to be somewhere between its own phenotype and the average phenotype of its family. Information of the second kind makes the family average useful negatively as a means of correcting partially for some nongenetic circumstances which affected the whole family. The net usefulness of the family average depends upon the balance between the two kinds of information it contains. When it is possible to reduce the C term to zero by proper experimental design, this makes the family average more useful than it would otherwise be.

The negative use of the family average can be illustrated by the problem of how to compare the breeding values of cows which made their records in different herds. Let each herd be considered as a "family" in which r has a value of .15 and t a value of .30 for butterfat production in a lactation. These are reasonable values, although uncertain enough to merit further investigation. The value used for t is close to what has been observed directly in several investigations. The value used for r is a bit higher than would be expected solely from relationship in the common case in which only one sire is used at a time, all females are born in the herd, and each sire is unrelated to his immediate predecessors. In herds containing 20 cows each, equation (4) becomes:

$$\hat{G}-\overline{G}=\frac{A}{A+C+E}\left\{\frac{17}{14}\ (P-\overline{P})-\frac{3}{14}\cdot\frac{200}{67}(Y-\overline{P})\right\}$$

If no attention were paid to the herd average the prediction equation would be: $\hat{G} - \overline{G} = \frac{A}{A + C + E} (P - \overline{P})$ as in mass selection. The difference can be written:

$$\frac{A}{A + C + E} \cdot \frac{3}{14} \left[(P - Y) - \frac{133}{67} (Y - \overline{P}) \right].$$

That is when the herd average is taken properly into account the cow is given a little positive credit for the amount by which her own record (P) exceeds her herd average (Y) and then is discounted rather more for the amount her herd average is above the general average of the whole population ($\overline{P}$). The reason for discounting the herd average is that the fact that t was larger than r is automatic proof that most herd averages are being shoved up or down by environmental circumstances such as feeding plan, conditions of pasture, health, etc. The proper negative use of the herd average automatically discounts these environmental effects on the cow's own record, as far as the information in t and r permits.

If equation (5) were used, it becomes:

$$\hat{G} - \overline{G} = \frac{A}{A + C + E} \left\{ \frac{17}{14} (P - \overline{Y}) + \frac{77}{134} (Y - \overline{P}) \right\}.$$

This gives the same numerical estimate for the cow but shows more clearly how much emphasis is put on the cow's own deviation $(P - Y)$ from her herd average and how much is put on the deviation $(Y - P)$ of her herd average from the general population average. The commonest practice in the United States to-day concerning dairy cows is to make no formal use of the herd average. This is equivalent to judging the cow wholly by $P - \overline{P}$. The "Stalldurchschnitt" or "byre average" method sometimes advocated in Europe, but perhaps not actually used widely, is equivalent to judging the cow wholly by $P - Y$. The best method, as shown above, is some kind of a compromise with more emphasis on $P - Y$ than is common to-day but not neglecting $Y - \overline{P}$ altogether. The exact point of compromise which is optimum in each case is determined by the sizes of r, t and n.

(To be concluded in September–October issue)

STUDIES ON BACTERIAL VARIATION AND ITS RELATION TO SOME GENERAL BIOLOGICAL PROBLEMS

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DURING recent work on certain problems of bacterial variation, results have been obtained which appear to have some interesting relations to a number of general biological problems, particularly to those of evolution and morphogenesis. The purpose of this communication, therefore, is to review and discuss the experimental results of work on dissociation in *Brucella abortus*, reported in detail elsewhere (Braun, 1943, 1945, 1946a, b),[1] and to point out certain parallels between these results and other biological problems.

While dissociation—*i.e.*, the appearance of variants within a bacterial population differing in colony form, culture characteristics, cell morphology, immunological reactions, biochemical reactions, virulence, etc.—has been noted from the beginning of experimental bacteriology, its interpretation has been varied and controversial. As early as 1912, Beijerinck suggested that bacterial variants are gene mutants. This view, however, found scant acceptance, particularly because the instability of bacterial variants did not appear to be comparable to the relative stability of mutants in higher animals and plants. Subsequently, Hadley (1927, 1937) interpreted dissociation as due to normal cyclogenic development of bacteria ("ontogenetic" theory), Henrici (1928) proposed the concept of "cytomorphosis," and Jollos (1924) applied the concept of "Dauer modifikationen" to bacterial variation.

The ideas of cyclogenic development underwent severe criticism when further experimental work demonstrated the lack of linked character variation during changes

[1] This work has been supported by the U. S. Bureau of Animal Industry.

from one type to another (*e.g.*, Mayer, 1938, Humphries, 1944). The latter workers, also others including Reed (1940) and Dubos (1945), have tried to explain the changes which occur during dissociation as being due to mutations, with a subsequent selection of mutants which can best persist in any given environment. Their views were strengthened when a number of geneticists started to focus their attention upon problems of genetics of microorganisms and provided substantial proof of the existence of undirected, spontaneous hereditary changes (mutations) in bacteria (Demerec, 1945; Demerec and Fano, 1945; Anderson, 1944; Luria and Delbrück, 1944; Gray and Tatum, 1944; Tatum, 1945). The here-to-be-reviewed experimental work with *Brucella abortus,* the causative agent of bovine infectious abortion, has now furnished data which permit a more complete understanding and interpretation of dissociation in terms of spontaneous appearance of variants (mutants) and their subsequent establishment within a population under the control of inherent and environmental factors governing population dynamics. (The data to follow will also indicate that the descriptive term "dissociation," introduced by de Kruif for bacterial variations involving smooth → rough changes and their accompanying changes in antigenicity, biochemical reactions, etc., is actually superfluous, since these phenomena can be explained as due to mutation and selection. It is, therefore, merely for the sake of compliance with established nomenclature of bacteriology that the term dissociation will be retained in the following discussion.)

Dissociation in *Br. abortus* involves changes from the antigenically active smooth (S) type to intermediate (I) and antigenically inactive rough (R), brown (Br) = mucoid, and other types. Differences between colonies of these types are striking when viewed on agar plates with the help of obliquely transmitted light. These differences make it easy to detect qualitatively and quantitatively the presence and degree of dissociation in a bac-

terial population. Thus, in most experiments, known amounts of one type of organisms, usually the S type, were inoculated into test-tubes with broth. After various periods, a loop-full of the broth-cultures was streaked on agar plates, and these plates were examined four days later. Since, as a rule, each cell gives rise to one colony, it was possible to estimate the degree of dissociation in a broth-culture by counting the percentage of dissociated and nondissociated colonies on an agar plate. Through such counts the "dissociation percentage" was determined, *i.e.*, the percentage of dissociated cells within a population after any given period of growth. For the "dissociation percentage" after 10 days of growth in broth, the term "dissociation index" has been chosen. Growth in broth usually resulted in the appearance of dissociated types after a few days. The dissociation percentage increased steadily until the cultures were approximately 12 days old; after that time it remained unchanged. (It was later determined that increase in dissociation percentage ceases at the time when growth stops in the culture.) Early in this work, it was noted that, even within a standardized environment (buffered broth at 37° C.), dissociation indices of strains from different sources differed significantly. This suggested the presence of inherent factors controlling dissociation percentages. By applying a new method of single cell isolation, it became possible to demonstrate the actual existence of such inherent factors. While under standard environmental conditions, the progeny from a single cell exhibited similar dissociation indices, the progeny from various cells showed dissimilar dissociation indices, *i.e.*, dissociation indices were constant for members of a clone. Dissociation indices differed significantly between clones, and it was possible to select systematically clones with different dissociation indices, ranging from a mean of less than 1 per cent. to a mean of 90 per cent. for respective clones. For example, a single cell was isolated, permitted to grow into a colony; this colony was streaked

onto another plate and the arising daughter colonies were individually inoculated into tubes with broth. Each broth culture was plated after 10 days and the percentage of dissociation in individual cultures was found to be 50 per cent., 52 per cent., 52 per cent., 54 per cent., 54 per cent., etc., for the progeny of one single cell, 7 per cent., 7 per cent., 7 per cent., 7 per cent., 9 per cent., etc., for the progeny of another single cell.

The availability of selected clones with known dissociation indices under standard conditions permitted studies on the modifying effects of a variety of environmental factors upon genetically controlled dissociation percentages, with the hope that such studies might lead to a better understanding of the nature of these inherent factors. It soon became evident that environmental conditions which alter population dynamics—*i.e.*, growth-rates and population pressure—modify dissociation percentages. Daily transfer of cultures, differences in nutrients, differences in temperature, differences in pH and reduced oxidation reduction potentials were found to change inherent dissociation percentages. (Under such altered environmental conditions, the absolute degree of dissociation is changed, but the relative differences between clones are retained, *i.e.*, environmental influences which lower the dissociation index will decrease the dissociation index of a high and low dissociating clone proportionally. For example, under standard environmental conditions the average dissociation index of clone "A" is 13 per cent., that of clone "B" 50 per cent.; in a medium with reduced $^{O}/_{R}$ potential, the dissociation index of "A" becomes 1 per cent., that of "B" 11 per cent.) Additional experiments provided substantial proof that dissociation does not occur without population growth (multiplication). Thus, dissociation percentages will remain stable when a broth culture has become approximately 12 days old, *i.e.*, when growth has ceased in such cultures; but if fresh broth is subsequently added, growth will recur and dissociation percentages will rise.

After a considerable amount of indirect evidence had been collected regarding the role of growth-rates and population pressure in the control of dissociation percentages, the necessary direct evidence of the relationship between population dynamics and dissociation was supplied by actual counts of bacteria. Two types of counts were made: (1) counts of the total number of bacteria per ml of broth were obtained by direct counting of

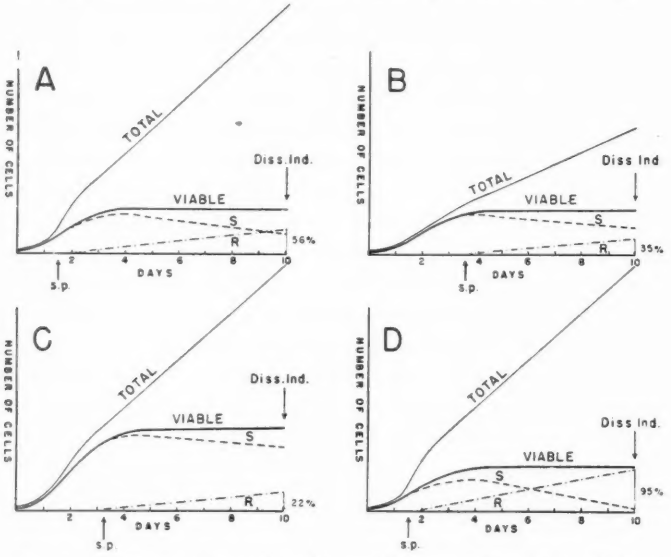

FIG. 1. Diagrams illustrating the establishment of variants within a population through spontaneous occurrence (mutation) and subsequent selection and the effects of viability and growth rate upon the dissociation index. A. basic diagram; B. slower growth rate of original type; C. higher viability of original type; D. faster growth rate, or higher viability of variant, or higher rate of appearance (higher mutation rate). For further details see discussion. s. p. = start of selection pressure; Diss. Ind. = Dissociation Index; - - - - - viable S cells; — · — · — viable R cells.

diluted samples in a counting-chamber using a microscope equipped with dark-field illumination; (2) counts of the number of living bacteria, more specifically the number of bacteria that have retained the ability of reproducing (called "viable" bacteria in Fig. 1) were made

by counting the number of colonies which grow on a plate streaked with known amount of diluted broth. Some general observations on population dynamics in bacteria were made first. It was found that, regardless of the size of the inoculum, the number of "viable" bacteria per ml soon reaches an optimum level (approximately 500 million per ml under "standard conditions"); if the inoculum is larger than this optimum number, a reduction in the number of "viable" bacteria will actually occur. The total number of cells, however, increases steadily as long as growth occurs (Fig. 1). The increase in the total number of cells per unit time is an indicator of the growth-rate of a clone. Comparison of the growth-rates of selected clones revealed inherent differences between growth-rates of clones with different dissociation indices; the clone with the highest dissociation index has the fastest growth-rate, clones with low dissociation indices exhibit slower growth-rates and longer duration of the lag phase. These and other experimental data have made it possible to show that dissociation percentages, as such, are not an inherent characteristic but are controlled by inherent factors which determine population dynamics.

Fig. 1 A illustrates the manner in which a variant (mutant) may establish itself within a population: during period of multiplication, a small percentage of mutants arise and any mutant with a higher selection value than the original members of the population can establish itself under conditions of selection pressure. Such conditions are quickly established within growing bacterial populations: namely, at the point where the total number of cells per ml becomes increasingly greater than the optimum number of "viable" cells. The difference between total number of cells and "viable" cells creates a *population* pressure which can act as *selection* pressure. However, the point at which this selection pressure starts is determined by the growth-rate and the optimum level of "viable" cells (viability) of the original members of a population and this, in turn, will determine dissociation

indices. Fig. 1 B illustrates what happens in a clone which has a slower growth-rate than the clone in Fig. 1 A. Selection pressure starts later in clone B than in clone A—consequently the dissociation index (after 10 days of growth) will be lower in clone B, although the percentage of arising mutants may be the same. The difference in growth-rate, controlled by inherent factors in clones A and B, can also be produced by environmental factors, like pH, and different dissociation indices for one clone can thus result under different environmental conditions. C in Fig. 1 illustrates how a shift in start of selection pressure and the subsequent effect on dissociation indices can result through higher "viability" of the original members of the population without change in growth-rates. Finally, Fig. 1 D illustrates the effect of changes in growth-rate or "viability" of the arising variant upon the dissociation index. The same graph, D, would also apply to changes in dissociation indices through changes in rate of appearance of variants (differential mutation-rates). This possible additional control of dissociation indices through differences in mutation rates can not be eliminated, although it has now been demonstrated how inherent differences or environmentally induced modifications of factors governing population dynamics can produce different dissociation indices even at equal mutation rates.

The apparent instability of bacterial mutants, the apparently specific adaptation of bacteria to their natural environment and their apparent ability to adapt themselves to altered environmental conditions are now easily explained. Even at mutation rates comparable to those of higher plants and animals, the existence of population pressure in bacterial populations will act as a constant selection pressure which permits the establishment of any mutant type which arises and has, in any given environment, a higher selection value than the original members of the population. The absence of sexual reproductions in bacteria will permit the immediate estab-

lishment of such mutants and their progeny and, due to the high reproduction rate of bacteria, such mutants can establish themselves very rapidly and replace the viable members of the original population. There is, therefore, no reason to assume that changes in characteristics of bacteria differ fundamentally from mutations in higher plants and animals.

The above cited results also are helpful for a better understanding of another widely observed phenomenon: namely, the apparent orderly successive mutational changes which have been noted in bacteria (*e.g.*, Hadley, 1937) and fungi (*e.g.*, Skovsted, 1943). Since, in the absence of sexual reproduction, the establishment of any mutant depends directly on its selection value (growth-rate, viability), it is obvious that, under uniform environmental conditions, as used in laboratory studies, the occurrence of apparently successive changes must be expected. For example, a morphologically different mutant B can establish itself in a population consisting of mutant A by virtue of B's higher selection value. Mutations to A may now occur in the B population, but since A has a lower selection value than B, they will be unable to establish themselves. However, mutant C arising in the B population may establish itself if it has a higher selection value than B. Thus, since in bacteria and most fungi, mutational steps must be judged by the *progeny* of one mutant which can establish itself, apparently successive changes A → B → C are observed. In cases where under identical environmental conditions a reversion from C → A is noted, it will be due to the appearance of a morphologically identical mutant A^1, which actually has a higher growth-rate and/or viability. Such morphologically identical mutants which differ in other characteristics have been observed during the work with *Brucella abortus*. Any changes in environment, of course, are expected to alter such successive changes because they alter the selection value of arising mutants.

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Further support for this concept has been provided by recent work on selective environments (Braun, 1946b). The addition of small amounts of antisera to broth cultures (as little as 2 per cent.) selectively restricts the ability of a mutant to establish itself within an environment which otherwise would favor its survival. Antisera for the R mutant thus completely prevent the occurrence of R types in an S clone which normally would show 70 per cent. R types after 10 days of growth. Actually, it was found that normal serum or plasma of rabbits, cows or hogs contains factors against the R and Br types, the most common type of variants from S. The addition of normal serum to broth cultures, therefore, prevents dissociation, *i.e.*, the establishment of these variants. In a very small percentage of cultures, dissociation will occur despite the presence of serum, but the variants which establish themselves in such cultures differ from those usually observed. They apparently represent mutants which normally have little chance to establish themselves when they are in competition with the R and Br types which have a higher selection value than these uncommon types.

The factors in normal serum which prevents the establishment of R and Br variants are not identical with other components (complement) of normal serum or plasma which exert a general bactericidal effect. Addition of normal serum in amounts used to prevent the establishment of dissociated types does not affect the growth-rate; furthermore, filtered or heated sera still possess the same action as unheated sera, while the factors causing the bactericidal effect are removed by filtration or heating.

It has long been known that if a mixture of S and R organisms is inoculated into an animal only the S type can be subsequently isolated. The demonstration of the presence in normal blood of factors inhibiting R and Br types now provides an explanation for this observation. It also indicates possibilities of a new therapeutic approach. In this approach, now under investigation,

efforts are being made to isolate the factor responsible for the *in vivo* inhibition of R and Br variants. Upon isolation of this factor it will be attempted to inactivate it *in vivo*, thereby altering the *in vivo* condition so that the establishment of avirulent types is favored. The avirulent R and Br types rapidly replace the S type *in vitro* in the absence of the serum factors and the same may be expected to take place *in vivo* if the serum factors are inactivated. In this connection some recent results by Watson (1946) are of interest. He has been able to isolate an anthracidal substance from the blood of animals which showed different degrees of normal resistance to *anthrax* infections. The substance proved to be of histone-like nature, with an unusually high lysine content, and was generally present in greater quantities in animals with high natural immunity. The substance is inactivated by thymonucleic acid but not by ribonucleic acid, a point which may be of general biological significance. But of greatest significance to the present discussion is the fact that the virulent *anthrax* type is a rough type and that, therefore, the substance isolated by Watson is a normally occurring compound affecting a rough type, which may possibly be identical with or similar to the normally occurring factors against R types of *Brucella*.

It will now also be possible to create specifically selective environments permitting the establishment of any one chosen mutant only, through the use of antiserum against a number of mutants, which before addition to broth-cultures have been absorbed with any one chosen mutant. This, in turn, will permit the study of such inherent characteristics as growth-rate and "viability," mutation-rate, etc., of any mutant in the absence of competing mutant types as well as in the presence of any desired combination of other mutants. Such studies, especially when combined with other immunological methods (*i.e.*, production of antibodies *in vitro*, Rosenow, 1945) and biochemical methods (Avery's work and

Tatum's studies are examples of the possibilities of the biochemical approach) should help to make bacteria a valuable tool in the investigation of problems of genetics and evolution.

An interesting parallel exists between the results so far obtained and certain evolutionary concepts as advanced by Wright (1937). Wright showed that the gene frequency, *i.e.*, the accumulation of small mutations, must depend on the size of the breeding population. He pointed out that among two geographically isolated groups, the group with the smaller genetically effective (breeding) population would have a higher gene frequency. A look at Fig. 1 A and C will show that this is exactly expressed in the experiments on bacterial variation here reviewed. For the curve of "total number of cells" read "number of generations per time unit"; for "viable cells" read "size of breeding population"; for "dissociation index" read "gene frequency", and Figs. 1 A and C will express Wright's particular evolutionary consideration. Similarly, Figs. 1 B and D can be translated into evolutionary principles, which will be much more obvious, however, than the one just mentioned. They are, for Fig. 1 B: at equal mutation-rates and equal size of breeding populations, the gene frequency will be higher in a group which has a greater number of generations per unit time; for Fig. 1 D: in populations with equally large breeding populations and the same number of generations per unit time differences in mutation-rates will alter the gene frequency. In this connection, it is interesting to note that Dobzhansky (1941) expressed the belief that "there are valid reasons to think that bacteria may prove to be the best available material for exact studies on mutation and natural selection."

Finally, some speculative thoughts may be expressed in regard to possible parallels between the principles of bacterial variation as exemplified above and some principles of morphogenesis. These thoughts concern pri-

marily the possible role of competition and selection in morphogenesis. Despite the recognized difficulty of fitting certain information, particularly results of work on plants and the development of protista into such ideas, a brief discussion may be justified by the stimulating conclusion which can be arrived at for one specific and important problem of morphogenesis: namely, the cancer problem. Others (*e.g.*, Henrici, 1928) have previously suggested possible parallels between tissue and the behavior of bacterial cells within a test-tube. If such parallels are applicable to the above discussed results, it could be inferred that competition plays a role in morphogenesis similar to the establishment of one predominant (and, provided the selective value of one type is sufficiently high, eventually *only*) type in a bacterial population where R and S types compete. The step which would lead to competing, differently differentiated, cells in morphogenesis may be in the nature of somatic mutations or, as Wright (1945) has suggested, controlled mutations of plasmagenes. Recently, some evidence has been supplied that mutations in bacteria can be controlled by the substrate (Dickinson, 1945); a similar control might restrict the direction of somatic mutations in morphogenesis. Experimental evidence concerning quantitative control of somatic mutations is also being obtained in some interesting current work with maize by Dr. B. McClintock.[2] (The concept of competition in morphogenesis was originally suggested by Roux, 1895; was advanced, among others, by Holmes, 1939; has entered into considerations of Goldschmidt, 1940, and has been the subject of a recent analysis by Spiegelman, 1945). Such speculations can, finally, be applied to the cancer problem. In the case of neoplasms, growth generally occurs in fully differentiated, non-growing tissue, *i.e.*, in the absence of such competitive forces as may normally exist during morphogenesis. Thus, after initiation of renewed growth, possibly through somatic muta-

[2] Unpublished results.

tions, the absence of such competitive forces in an adult organism may be responsible for the unchecked growth or its failure to adjust into regulated, *i.e.*, harmonically differentiated, tissue. By transplanting neoplastic cells into an undifferentiated embryonic environment of the same animal it should be possible to test whether this absence of competition plays a role in the genesis of cancer and whether neoplastic cells become harmonically differentiated when subjected to selective competition presumably existent in an actively growing embryonic environment.

LITERATURE CITED

Anderson, E. H.
1944. *Proc. Nat. Acad. Sci.*, 30: 397–403.
Avery, O. T., C. M. MacLeod and M. McCarty
1944. *Jour. Exp. Med.*, 79: 137–158.
Beijerinck, M. W.
1912. *Folia Microbiologica*, 1: 377.
Braun, W.
1943. *Jour. Bact.*, 45: 21–22.
1945. *Science*, 101: 182–183.
1946a. *Jour. Bact.*, 51: 327–349.
1946b. *Jour. Bact.*, 52: 243–249.
Demerec, M.
1945. *Proc. Nat. Acad. Sci.*, 31: 16–24.
Demerec, M. and V. Fano
1945. *Genetics*, 30: 119–136.
Dickinson, L.
1945. *Jour. Path. and Bact.*, 57: 285–294.
Dobzhansky, Th.
1941. "Genetics and the Origin of Species." 2nd Ed. New York: Columbia Univ. Press.
Dubos, R.
1945. "The Bacterial Cell." Cambridge, Mass.: Harvard University Press.
Goldschmidt, R.
1940. "The Material Basis of Evolution." New Haven, Conn.: Yale Univ. Press.
Gray, C. H. and E. L. Tatum
1944. *Proc. Nat. Acad. Sci.*, 30: 404–410.
Hadley, P.
1927. *Jour. Infectious Diseases*, 40: 1–312.
1937. *Jour. Infectious Diseases*, 60: 129–192.
Henrici, A. T.
1928. "Morphologic Variation and the Rate of Growth of Bacteria." Springfield, Ill.: Charles C Thomas, Publisher.

Holmes, S. J.
1939. *The Scientific Monthly*, 59: 253–260.
Humphries, J. C.
1944. *Yale Jour. Biol. Med.*, 16: 639–658.
Jollos, V.
1924. *Centr. f. Bakt.* I, 93: 22–49.
Luria, S. E. and M. Delbrück
1943. Genetics, 28: 491–511.
Mayer, H. D.
1938. "Das 'Tibi'—Konsortium nebst einem Beitrag zur Kenntnis der Bakterien—Dissoziation." Dissertation, Druk Naamloze Vennotschap W. D. Meinema, Delft, Holland.
Reed, G. B.
1940. Pub. Am. Assoc. Advancement Sci., No. 12: 28–33.
Rosenow, E. C.
1945. *Jour. Infectious Diseases*, 76: 163–178.
Roux, W.
1881. "Der Kampf der Theile im Organismus." Leipzig: Verlag von Wilhelm Engelmann.
Skovsted, A.
1943. *Comptes rendus d. Labor. Carlsberg, Ser. physiol.*, 23: 409–456.
Spiegelman, S.
1945. *Quart. Rev. of Biol.*, 20: 121–146.
Tatum, E. L.
1945. *Proc. Nat. Acad. Sci.*, 31: 215–219.
Watson, D.
1946. *Jour. Bact.*, 51: 643.
Wright, S.
1937. *Proc. Nat. Acad. Sci.*, 23: 307–320.

CHROMOSOMES OF DIGENETIC TREMATODES[1]

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INTRODUCTION

THE Digenea, a subclass of the Trematoda, is composed of a group of parasitic worms whose relationships to one another are not yet certain. Much of the uncertainty may be attributed to the fact that nearly all the major and minor groups have been established on the basis of gross morphological characters in the adult. The complicated life histories of these worms seem to make it necessary to consider other characters, such as larval and cytological, as well, in attempting to show relationships within the group. Accordingly, Cort (1917), Faust (1919), La Rue (1926) and others have suggested as significant the characteristics of the excretory system and larval stages in establishing criteria that might be relied upon for demonstrating phylogenetic relationships. More recently Talbot (1933 and 1934), Byrd (1935) and McMullen (1935b, 1935c and 1937) have produced evidence, based on a study of larval characters, that demonstrates an apparent relationship between certain members of this group. This line of investigation, however, has not been applied extensively, and other approaches to the problem, such as the cytological, have been neglected. Descriptions of spermatogenesis and oogenesis are found in the literature for 14 genera and 15 species of these hermaphroditic worms (Table 1), but heretofore no investigation has dealt primarily with the chromosomes. As will be shown below, chromosome studies can contribute much to an understanding of species delimitation and reveal those numerical and structural changes that

[1] I wish to thank Drs. Ladley Husted and B. D. Reynolds, of the Miller School of Biology, University of Virginia, for suggestions and criticisms offered throughout the investigation. Thanks are also due Mr. Leslie Burger for collecting many of the hosts.

have accompanied the development and diversification of the group.

Thirty-five species (Table 1), representing 25 genera and 8 families, were studied with regard to chromosome number, morphology and behavior. Each species has a constant chromosome number, but variations within genera and families occur. Chromosome numbers[2] of 7, 8, 9, 10, 11 and 14 occur in species of the families invesgated. As in another group of hermaphroditic parasites, the cestodes (Jones, 1945), and in the free-living hermaphroditic pulmonate gastropods (Husted and Burch, 1946) evidence of polyploidy is lacking. Aneuploidy, however, appears to have played a part in speciation in this group.

Materials and Methods

All trematodes, except two species, were taken from cold-blooded hosts, chiefly reptiles. These were killed by bleeding and the parasites removed immediately, rinsed in normal saline, and fixed by Looss' shaking method in one of the three fixatives described below. A list of the hosts and the number of parasites taken from each species is presented in Table 1.

A series of 18 fixatives[3] were tested on the trematode *Plagitura salamandra* for the purpose of selecting the one most satisfactory for the results desired. Carnoy 6:3:1 for two hours proved to be the most reliable. Hot Bouin for 12 hours gave results comparing favorably with Carnoy. Material to be stained by means of the Feulgen reaction was satisfactorily fixed by treating with Carnoy for five minutes followed with Sanfelice for two hours. All material was removed from the fixative, washed in several changes of 85 per cent. alcohol and stored in 70 per cent. ethyl alcohol.

[2] Unless otherwise stated these refer to genetic numbers.

[3] Petrunkevitch, Sanson, Carnoy-Lebrun, Gilson, Champy, Bouin (cold) LaCour 2BE and 2BD, Helly, Carnoy plus osmic acid, Carnoy followed with Sanfelice, Randolph, Medium and Strong Flemming, Zacharia, Smith, Hot Bouin, Carnoy and Sanfelice.

Material for sectioning was prepared by passing through the various percentages of ethyl alcohol, clearing in chloroform and embedding in paraffin. Sections were cut at 12 microns and stained on the slide with Heidenhain's iron hematoxylin or crystal violet. Iron hematoxylin gave the best results. Following "double fixation" in Carnoy and Sanfelice, a hydrolysis time of 25 minutes and stain for two hours gave a satisfactory Feulgen reaction. *Toto* preparations, for identification purposes, were stained with Reynolds' Delafield-Cochineal for two hours and destained in 45 per cent. acetic acid.

Meiotic chromosomes were studied in both spermatocytes and oocytes; mitotic chromosomes at first cleavage.

All drawings were made at table level with the aid of a camera lucida and are reproduced at a magnification of —×. The idiograms were constructed from measurements of mitotic chromosomes and represent only the four longest pairs in the complement.

Observations and Discussion

Mitotic chromosomes are best observed during the first cleavage of the zygote. Here the chromosomes are less crowded than in other cells due to the larger size of the cell and spindle. They can also be observed in cells of the ovary, testes and developing embryo, but the spindles here are usually too small to permit accurate analysis of the complement.

Meiotic chromosomes are readily observed in the cells of the gonads. All phases of the maturation of the male sex cells occur in the testes and in each of the 35 species studied the process is the same. There are three spermatogonial divisions followed by two maturation divisions. The cells remain attached, following each division, resulting in a rosette-like structure consisting of 32 cells. Each of these cells give rise to a thread-like sperm. Maturation of the female sex cells begins in the ovary and is completed in the upper portion of the uterus,

after the entrance of the sperm and the formation of the shell. The early prophase stage, which takes place in the ovary, is followed by a diffuse period which continues until the oocyte enters the uterus, where it receives the sperm. As a rule bivalents at diakinesis are not observed in primary oocytes until after the entrance of the sperm. Occasionally, however, the chromosomes condense precociously while the oocyte is in the ovary, as was observed in *Allocreadium isoporum* (Fig. 1) and *Bunodera luciopercae*. Markell (1943) reports the same in *Probolitrema californiense*.

On a comparative basis the chromosomes of each complement exhibit a considerable range in size. The largest chromosomes occur in the family Allocreadiidae (Figs. 1, 2, 3, 4 and 39) where some pairs measure up to 8 microns in length. The smallest occur in the families Clinostomidae (Fig. 8) and Plagiorchiidae (Figs. 16, 17 and 45).

The number of chromosomes in a complement is constant for each species but between genera and species there is variation. Numbers of 7, 8, 9, 10, 11 and 14 are present in the various species studied (Table 1). The chiasma frequency is relatively high in all species, the number per bivalent being approximately proportional to the length of the chromosomes. As many as 14 were observed in a long pair of chromosomes in *Crepidostomum serpentinum*. In the shorter chromosomes as few as one chiasma per bivalent occurs but in no case were univalents observed.

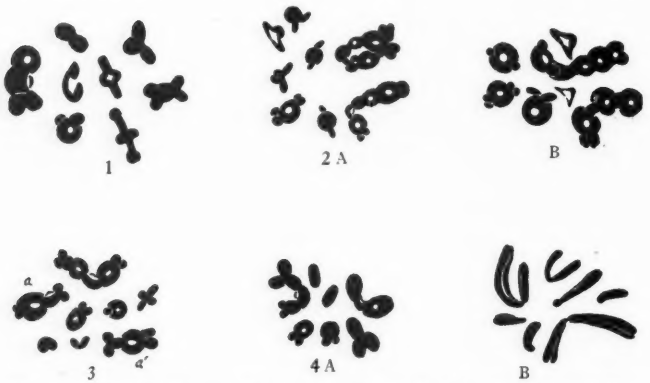
The Allocreadiidae (Figs. 1–4): Four species, representing three genera of the family, are included here. Large chromosomes are characteristic of all four species examined and possibly characteristic of the family as a whole. On the basis of chromosome morphology the species are distinct. Each has at least two long pairs of chromosomes and one, *Bunodera saculata*, has three. It is of some interest that in the species with three long pairs two of the bivalents are so similar that they appear

TABLE I

Family and Species	Specimens		Chromosome No.		Author	Host
	Collected	Examined	n	2n		
PRONOCEPHALIDAE						
Macrovestibulum kepneri			9	18	Jones *et al.*, 1946	
PARAMPHISTOMIDAE						
Diplodiscus temporatus			8	16	Cary, 1909	
FASCIOLIDAE						
Fasciola hepatica			6	12	Henneguy, 1902 Schubmann, 1905 Schellenburg, 1911	
ZOOGONIDAE						
Zoogonus mirus			5	10	Goldschmidt, 1905	
			6	12	Schreiner, 1908 Gregoire, 1909 Wassermann, 1913	
DICROCOELIIDAE						
Dicrocoelium lanceolatum			10	20	Goldschmidt, 1908 Dingler, 1910	
BRACHYCOELIIDAE						
Brachycoelium salamandrae			10	20	von Kemnitz, 1913	
HETEROPHYIDAE						
Cryptocotyle lingua			6	12	Cable, 1931	
ECHINOSTOMIDAE						
Parorchis acanthus			11	22	Rees, 1939	
BUCEPHALIDAE						
Bucephalus elegans			6	12	Woodhead, 1935	
B. pusillus			6	12	Woodhead, 1935	
TROGLOTREMATIDAE						
Paragonimus kellicotti			8	16	Chen, 1937	
AZYGIIDAE						
Proterometra macrostoma			9	18	Anderson, 1935	
Azygia acuminata	2	1	9	18	Britt	*Amia calva*
ALLORCEADIIDAE						
Allocreadium isoporum	6	3	8	16	Britt	*Morone americana*
Crepidostomum serpentinum	200	10	8	16	Britt	*Natrix septemvittata*
Bunodera saculata	2	1	8	16	Britt	*Perca flavescens*
B. luciopercae	1	1	7	14	Britt	*Perca flavescens*
CLINOSTOMIDAE						
Clinostomum marginatum	3	2	10	20	Britt	*Ardea herodias*
LECITHODENDRIIDAE						
Loxogenes bicolor	10	5	11	22	Britt	*Rana clamitans*
Acanthatrium pipistrella	15	9	11	22	Britt	*Myotis sp.*

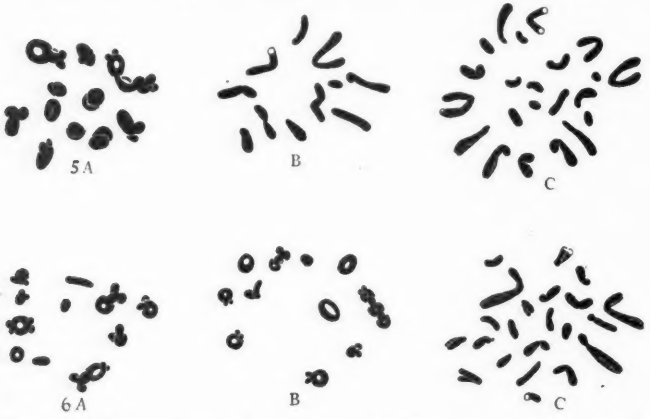
TABLE I—(*Continued*)

Family and Species	Specimens		Chromosome No.		Author	Host
	Collected	Examined	n	2n		
CEPHALOGONIMIDAE						
Cephalogonimus americanus	1	1	14	28(?)	Britt	*R. clamitans*
GORGODERIDAE						
Probolitrema californiense			6	12	Markell, 1943	
Gorgoderina attenuata	3	2	7	14	Britt	*R. catesbiana*
Gorgodera amplicava	1	1	8	16	Britt	*R. clamitans*
PLAGIORCHIIDAE						
Eustomas chelydrae	20	6	9	18	Britt	*Chrysemys picta*
Glyphelmins quieta	2	1	9	18	Britt	*R. catesbiana*
Plagitura salamandra	75	21	11	22	Britt	*Triturus viridescens*
Pneumobites breviplexus	2	1	11	22	Britt	*R. clamitans*
Pneumococces medioplexus			11	22	Pennypacker, 1936	
P. similiplexus			11	22(?)	Pennypacker, 1940	
P. similiplexus	2	2	11	22	Britt	*R. pipiens*
RENIFERIDAE						
Natriodera verlatum	35	10	11	22	Britt	***Natrix sipedon***
Dasymetra villicoeca	60	10	11	22	Britt	*N. sipedon*
Pneumatophilus leidyi	30	10	11	22	Britt	*N. sipedon*
P. variabilis	150	10	11	22	Britt	*N. sipedon*
Lechriorchis abduscens	4	2	11	22	Britt	*Lampropeltis getulus getulus*
Renifer ellipticus	14	8	11	22	Britt	*Agkistrodon piscivorous*
Neorenifer wardi	20	10	11	22	Britt	*N. sipedon*
N. georgianus	80	20	11	22	Britt	*Coluber constrictor con.*
N. antarum	300	10	11	22	Britt	*N. sipedon* and *L. getulus g.*
N. orula	20	10	11	22	Britt	*N. sipedon*
N. drymarchon	6	4	11	22	Britt	*Drymarchon corais*
N. elongatus	6	2	11	22	Britt	*C. constrictor constrictor* & *L. getulus getulus*
Staphylodora bascaniensis	7	4	8	16	Britt	*C. constrictor constrictor*
Auridistomum chelydrae	25	10	9	18	Britt	*Chelydra serpentina*
Telorchis robustus	100	10	8	16	Britt	*Cistudo carolina*
T. lobusus	50	10	11	22	Britt	*C. serpentina*
T. medius	5	2	11	22	Britt	*Kinosteron subrubrum*
T. corti	9	5	11	22	Britt	*C. serpentina*



Figs. 1–4, Family Allocreadiidae: (1) *Allocreadium isoporum*, meiotic prometaphase, ovary, n = 8; (2a) *Crepidostomum serpentinum*, met. I, testis, n = 8; (b) met. I, egg, n = 8; (3) *Bunodera saculata*, met. I, testis, n = 8; (4a) *B. luciopercae*, met. I, testis, n = 7; (b) met. II, egg, n = 7.

to be duplicates (Fig. 3; a and a′). This, along with the fact that a second species of the genus (*B. luciopercae*) has only 7 chromosomes, while *B. saculata* has 8, suggests that the latter could be an aneuploid species. *Allocreadium isoporum* and *Crepidostomum serpentinum*,



Figs. 5–6, Family Lecithodendriidae: (5a) *Loxogenes bicolor*, met. I, testis, n = 11; (b) met. II, egg, n = 11; (c) Mitotic met., egg, 2n = 22; (6a) *Acanthatrium pipistrella*, prometaphase, I, testis, n = 11; (b) Prometaphase I, egg, n = 11; (c) Mitotic met., egg, 2n = 22.

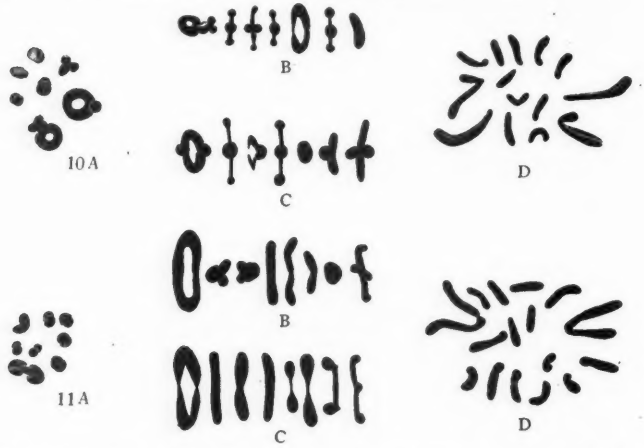
each with 8 chromosomes, are placed in the subfamily Allocreadiinae. The genus *Bunodera* is assigned to the subfamily Bunoderinae.

THE LECITHODENDRIIDAE (Figs. 5–6): The family Lecithodendriidae contains species from both cold- and warm-blooded hosts. A single representative from each type of host was examined—*Loxogenes bicolor* from the intestinal tract of the frog and *Acanthatrium pipistrella* from the intestinal tract of the little brown bat. On the basis of chromosome number the two genera are similar in

Figs. 7–9, Families Azygiidae, Clinostomatidae and Cephalogonimidae: (7a) *Azygia acuminata*, met. I, testis, n 9; (b) Met. I, egg, n = 9); (c) Met. II, egg, n = 9; (d) Mitotic met., egg, 2n = 18; (8) *Clinostomum marginatum*, met. I, testis, n = 10; (9) *Cephalogonimus americanus*, prometaphase I, egg, n = 14.

that each has a chromosome number of 11. On the basis of chromosome morphology the genera are distinct. *Loxogenes bicolor* has a single pair of long chromosomes, while *A. pipistrella* has two long pairs. *Loxogenes bicolor* has five pairs of medium-long chromosomes and *A. pipistrella* only four. Each has five pairs of small chromosomes. The similarity in chromosome number and morphology of these species to species of the Plagiorchiidae and Reniferidae may indicate a close relationship, as was suggested by McMullen (1937).

THE AZYGIIDAE (Fig. 7), CLINOSTOMATIDAE (Fig. 8) and CEPHALOGONIMIDAE (Fig. 9): These three families are each represented here by a single species. *Azygia acuminata* (Azygiidae) has 9 chromosomes, the same number reported by Anderson (1935) for *Proterometra macrostoma* which belongs to the same family. *Clinostomum marginatum* (Clinostomatidae) has a chromosome count of 10 and the chromosomes are among the smallest observed for any species. *Cephalogonimus americanus*

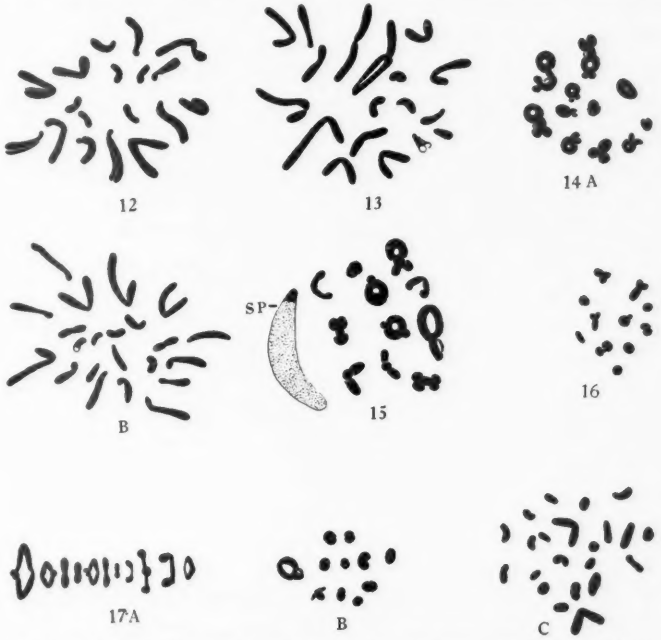


Figs. 10–11, Family Gorgoderidae: (10a) *Gorgoderina attenuata*, met. I, testis, n = 7; (b) Met. I, egg, n = 7; (c) Met. I, testis, n = 7; (d) Mitotic met., egg, 2n = 14; (11a) *Gorgodera amplicava*, met. I, testis, n = 8; (b) Met. I, egg, n = 8; (c) Met. I, testis, n = 8; (d) Mitotic met., egg, 2n = 16.

(Cephalogonimidae), on the basis of an analysis of prometaphase chromosomes in a single primary oocyte, has 14 chromosomes. This is the highest number reported for any trematode, but the determination must be considered tentative until more material is examined.

THE GORGODERIDAE (Figs. 10–11): Chromosome numbers are known for three genera and species of the Gorgoderidae—*Probolitrema californiense* 6 (Markell, 1943), *Gorgoderina attenuata* 7, and *Gorgodera amplicava* 8. On the basis of differences in chromosome number and

morphology the three genera are distinct. *Gorgoderina attenuata* has two pairs of long chromosomes (Figs. 10a–10d and 37), while *G. amplicava* has only a single pair (Figs. 11a–11d and 38). These two genera are quite similar morphologically and are separated chiefly on the



Figs. 12–17, Family Plagiorchiidae: (12) *Eustomas chelydrae*, mitotic met., egg, 2n = 18; (13) *Glythelmins quieta*, mitotic met., egg, 2n = 18; (14a) *Plagitura salamandra*, met. I, testis, n = 11; (b) Mitotic met., egg, 2n = 22; (15) *Pneumobites similiplexus*, prometaphase I, egg, n = 11 sp = sperm; (16) *P. breviplexus*, Met. I, testis, n = 11; (17a) *Pneumonoeces medioplexus*, met. I, testis, n = 11; (b) Met. I, egg, n = 11; (c) Mitotic met., egg, 2n = 22.

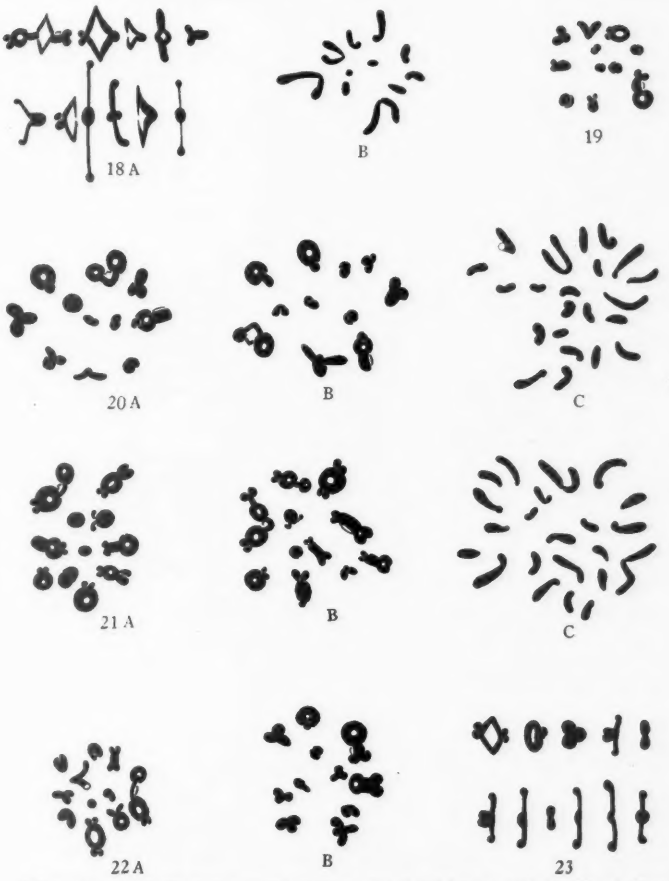
basis of a difference in the number of testes. The difference in chromosome number supports their being separated into two different genera.

The Plagiorchiidae (Figs. 12–17): The family as defined at present includes a diverse assortment of trematodes that are grouped into several subfamilies. Five

genera—*Eustomas, Glypthelmins, Pneumonoeces, Pneumobites* and *Plagitura*—are represented here. The first two have 9 chromosomes; the others 11. On the basis of number and size the chromosomes of *Eustomas chelydrae* (Fig. 12) and *Glypthelmins quieta* (Fig. 13) are similar. However, the two genera may be separated on the difference in chromosome morphology (Figs. 42 and 43). Those two genera are placed in the subfamily Plagiorchiinae. *Pneumobites similiplexus, P. breviplexus* and *Pneumonoeces medioplexus,* all with 11 chromosomes and similar morphology (Figs. 15–17) are grouped together in the subfamily Saphederinae. *Plagitura salamandra* (Fig. 14), although having the same number of chromosomes (11) as do the latter species, has a complement that resembles more closely those of *E. chelydrae* and *G. quieta* than *Pneumobites* and *Pneumonoeces.* This may indicate a closer relationship of *Plegitura* to the former genera than to the latter.

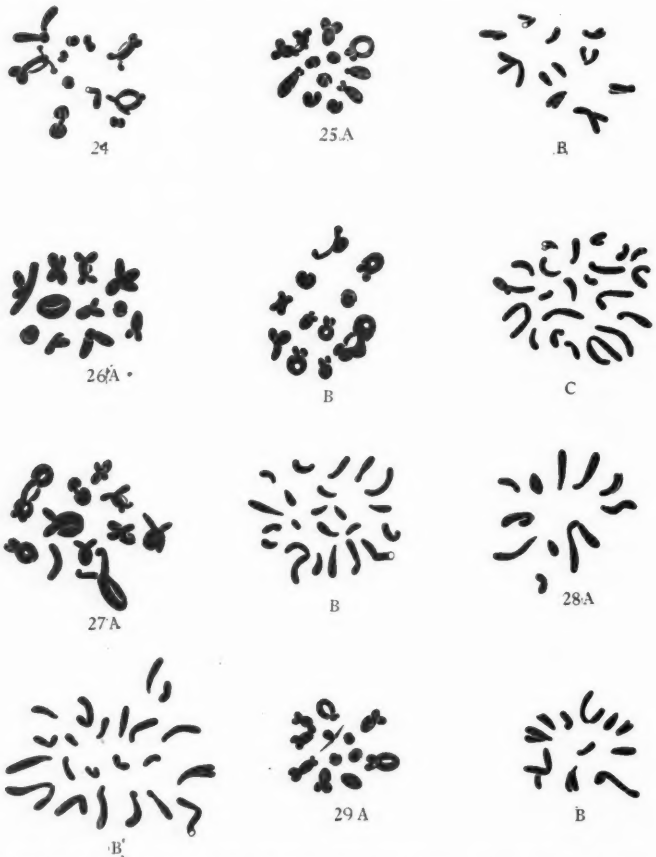
The Reniferidae (Figs. 18–35 and 46–56): The family as now defined is divided into five subfamilies. Of the 18 species investigated 12 are assigned to the subfamily Reniferinae, 1 to the Staphylodorinae and 5 to the Telorchiinae. Cytologically the 12 species (6 genera) assigned to the Reniferinae are strikingly similar. Each species has 11 chromosomes, with at least one long pair in the complement (Figs. 18–29 and 46–52). *Natriodera verlatum* has two long pairs while all the other species of the subfamily have a single pair. The chromosomes of *Pneumatophilus variabilis* (Figs. 20c and 47) and *P. leidyi* (Figs. 21c and 48) are so similar that the question may be raised, on cytological grounds, as to whether *P. leidyi* is a distinct species.

Staphylodora bascaniensis (Figs. 30 and 53), the only representative of the subfamily Staphylodorinae examined, differs cytologically from the genera assigned to the former subfamily chiefly in chromosome number. The number is 8. Although differing in number and size from the chromosomes of *Natrodera verlatum* (Fig.



Figs. 18–23, Subfamily Reniferinae (Reniferidae): (18a) *Natriodera verlatum*, met. I, testis, n = 11; (b) Met. II, egg, n = 11; (19) *Dasymetra villicaeca*, met. I, testis, n = 11; (20a) *Pneumatophilus variabilis*, met. I, testis, n = 11; (b) Met. I, egg, n = 11; (c) Mitotic met., 2n = 22; (21a) *P. leidyi*, met. I, testis, n = 11; (b) Met. I, egg, n = 11; (c) Mitotic met., egg, 2n = 22; (22a) *Lechriorchis abdusccns*, met. I, testis, n = 11; (b) Met. I, egg, n = 11; (23) *Renifer ellipticus*, met. I, testis, n = 11.

18b) there is a similarity in morphology. With respect to number and morphology the chromosomes of this species also resemble those in *Telorchis robustus*, of the subfamily Telorchiinae (Figs. 31c and 54).



Figs. 24–29, Subfamily Reniferinae (Reniferidae): *Neorenifer elongatus*, met. I, testis, n = 11; (25a) *N. georgianus*, met. I, testis, n = 11; (b) Met. II, egg, n = 11; (26a) *N. wardi*, met. I, testis, n = 11; (b) Met. I, egg, n = 11; (c) Mitotic met., egg, 2n = 22; (27a) *N. aniarum*, met. I, testis, n = 11; (b) Miottic met., egg, 2n = 22; (28a) *N. orula*, met. II, egg, n = 11; (b) Mitotic met., 2n = 22; (29a) *N. drymarchon*, met. I, testis, n = 11; (b) Met. II, egg, n = 11.

The subfamily Telorchiinae is represented here by five species. Three species, *Telorchis* (*medius, lobosus* and *corti*), have chromosome numbers of 11 while a fourth, *T. robustus*, has only 8. *Auridistomum chelydrae*, an-

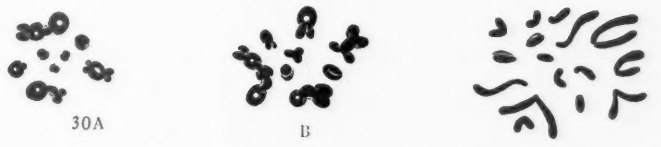
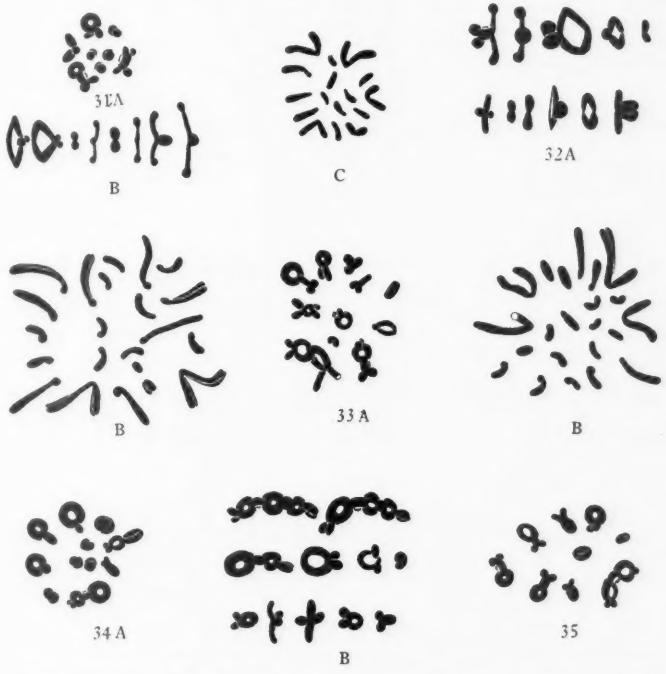
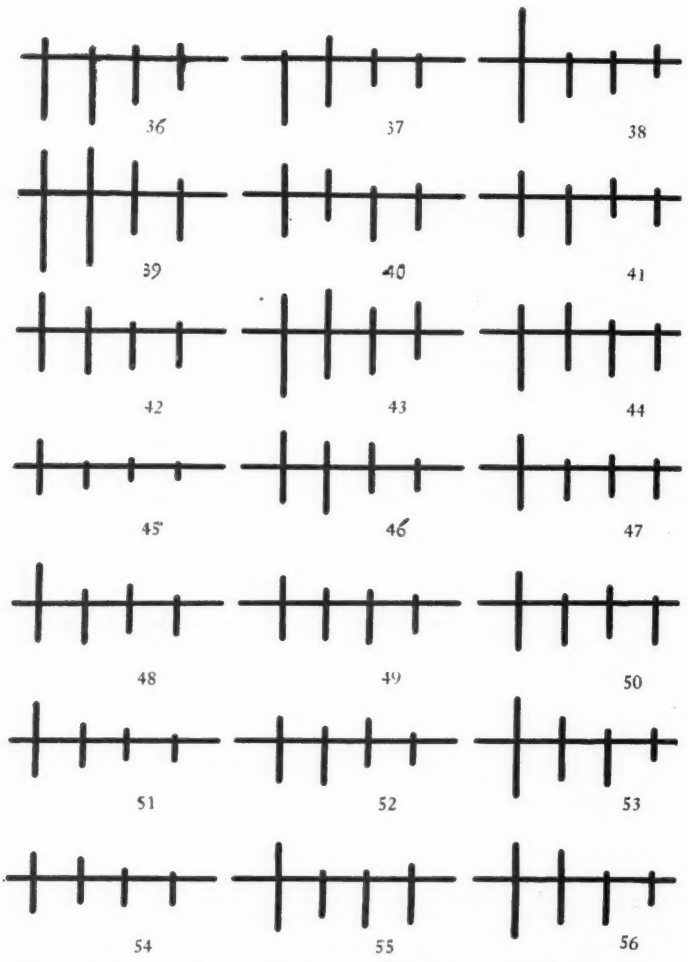


Fig. 30, Subfamily Staphylodorinae (Reniferidae); (a) *Staphylodora bascaniensis*, met. I, testis, n = 8; (b) Met. I, egg, n = 8; (c) Mitotic met., egg, 2n = 16.

other member of the subfamily, has 9. Similarities in chromosome complements between species of the sub-



Figs. 31–35, Subfamily Telorchiinae (Reniferidae); (31a) *Telorchis robustus*, met. I, testis, n = 8; (b) Met. I, egg, n = 8; (c) Mitotic met., egg, 2n = 16; (32a) *T. lobosus*, met. I, egg, n = 11; (b) Mitotic met., egg, 2n = 22; (33a) *T. medius*, met. I, testis, n = 11; (b) Mitotic met., egg, 2n = 22; (34a) *T. corti*, met. I, testis, n = 11; (b) Prometaphase I, testis, n = 11; (35) *Auridistomum chelydrae*, met. I, testis, n = 9.



Figs. 36–56, Idiograms: (36) *Azygia acuminata;* (37) *Gorgoderina attenuata;* (38) *Gorgodera amplicava;* (39) *Bunodera luciopercae;* (40) *Loxogenes bicolor;* (41) *Acanthatrium pipistrella;* (42) *Eustomas chelydrae;* (43) *Glypthelmins quieta;* (44) *Plagitura salamandra;* (45) *Pneumonoeces medioplexus;* (46) *Natriodera verlatum;* (47) *Pneumatophilus variabilis;* (48) *P. leidyi;* (49) *Neorenifer wardi;* (50) *N. orula;* (51) *N. aniarum;* (52) *N. drymarchon;* (53) *Staphylodora bascaniensis;* (54) *Telorchis robustus;* (55) *T. lobosus;* (56) *T. medius.*

families Reniferinae and Telorchiinae suggests a relationship between these two groups.

The number of families, genera and species represented in this study is not sufficiently large to warrant a grouping of families into superfamilies based on cytological characters. However, chromosome number and morphology constitute evidence which supports McMullen's (1937) inclusion of the Lecithodendriidae with the Reniferidae and Plagiorchiidae in the superfamily Plagiorchioidea. On the other hand, the chromosomes of the Allocreadiidae are so different (number and morphology) from those of the Plagiorchiidae and Reniferidae that it does not seem probable that these families are as closely related as Dollfus (1930) and Faust (1939) intimate.

In a majority of the families of the Digenea, for which chromosome numbers are known for more than a single species, there is a variation. The families include the Reniferidae, which has species with 8, 9 and 11 chromosomes; the Plagiorchiidae with 9 and 11; the Allocreadiidae with 7 and 8; and the Gorgoderidae with 6, 7 and 8. Considering the hermaphroditic Digenea as a group, chromosome numbers are known for 48 species representing 18 families (Table 1). These families contain species with 6, 7, 8, 9, 10, 11 and 14 chromosomes. The fact that such variation occurs indicates that changes in chromosome number have accompanied the diversification of these hermaphroditic organisms and suggests that such changes played a part in their evolution.

Polyploidy is almost unknown in the higher animals which, as suggested by Muller (1925), may be due to their bisexual condition. In groups of hermaphroditic animals there seems to be no reason why polyploidy should not be one of the methods of speciation since no sex-chromosome mechanism is involved (White, 1940). Nevertheless, there is but little evidence that it even occurs there. White examined all available data on

chromosome numbers in the Rhabdocoela, Oligochaeta, Hirudinea and Pulmonata and concluded that, based on the occurrence of species with multiple numbers, there were possible polyploids in the Rhabdocoela, Oligochaeta and Hirudinea but not in the Pulmonata. He concedes, however, that the number of actual polyploid species is probably less than the data indicate since species may have multiples of others without being polyploids. The data show a majority of the species with chromosome numbers that differ not by multiples but by progression. This suggests that these differences have come about not by a doubling of whole sets of chromosomes but by gradual additions or losses. The same situation appears to be true for other hermaphroditic animals such as the cestodes, the trematodes reported here and the pulmonate gastropods.

Jones (1945) determined the chromosome numbers for 15 species of tapeworms in the families Hymenolepidae and Diplepididae and reported numbers of 5, 6, 7 and 8. Not one of these has a chromosome number that is a multiple of another. However, the progressive increase from 5 to 8 suggests aneuploidy. Husted and Burch (1946) determined chromosome numbers for 18 species and subspecies of the Polygyridae (Pulmonata) and found no evidence for polyploidy, but what appears to be aneuploidy is evident. In *Triodopsis fraudulenta* they found individuals with 58, 59, 60 and 61 or 62 chromosomes. A mosaic of the same species contained cells with 62, 64 and 66. They suggest "that in the Pulmonata as a whole the progressive increase from 34 to 62 chromosomes may be attributed entirely to the duplication of chromosomes one or two at a time rather than a sudden doubling of the complement and subsequent loss. . . . With the intervention of a period of adjustment, . . ., a state may be established in time which approaches and is comparable to polyploidy." One can not overlook the possibility that species in other groups of hermaphroditic animals with multiple chromosome numbers may have

reached that condition through such a process and therefore are not necessarily polyploids but aneuploids with numbers which are incidently multiples of other species.

Considering the chromosome numbers of the hermaphroditic Digenea as a whole one species has a number which is a multiple of another and therefore could possibly be polyploid. The species is *Cephalogonimus americanus* and has a chromosome number of 14, which is double that of two other species—*Bunodera luciopercae* and *Gorgoderina attenuata*. However, there is too great a difference in chromosome size and morphology between these three species to suggest a close relationship. Besides, they are so different structurally that they are assigned to separate families. The progressive increase in chromosome numbers, which is apparent in other species of the group, and which appears to be characteristic for the Digenea, could account for the increased chromosome number in *C. americanus*. Therefore, *C. americanus* is not considered a polyploid.

The variation in chromosome numbers occurring in the Digenea, and in other groups of hermaphroditic animals, may be attributed to the gradual addition or loss of chromosomes. The presence of what appears to be a bivalent in duplicate (Fig. 3) and the fact that the Monogenea, from which the Digenea are believed to have evolved, have, as far as is known, low chromosome numbers[4] suggests that the change in numbers in the Digenea has taken place by the addition of chromosomes rather than by loss. Thus, the addition of chromosomes to the complement appears to have played a major role in the evolution of this group, notwithstanding the fact that it is generally believed that aneuploidy could have played no important part in evolution because of physiological unbalance brought about on the addition of incomplete sets of chromosomes. It can not be denied that available experimental data support such a conclusion but it does not

[4] *Polystomum integerrinum*, n = 4 (Goldschmidt, 1902); *Gyrodactylus elegans*, n = 6 (Gille, 1914).

necessarily follow that all, or even a majority, of the unbalances occurring in nature are inferior to "normal" combinations, for certain kinds of "balances" may equal or even be superior to the tried condition. As Darlington (1937) points out, there is always the possibility of variation occurring by a change in proportion due to the reduplication of whole chromosomes and that "If such a new proportion were successful it would yield a new secondary balance having a different phenotypic expression from the primary, ancestral, type." The number of successful combinations of "unbalanced" gametes is supposed to be few and the chances for survival of the new type in competition with the normal remote. However, the progressive increase in chromosome numbers, as exhibited in the hermaphroditic animals, indicates aneuploidy and suggests that successful unbalanced combinations occur more frequently than have been supposed. Just how the increases have come about is not evident but duplications in chromosome segments with subsequent breaks and recombinations could result in an increase in the number of chromosomes in a complement without greatly upsetting the balance. Such a process could easily account for the apparent great number of successful "unbalanced" combinations.

Summary

Thirty-five species of digenetic trematodes, representing 25 genera and 8 families, have been studied with regard to chromosome number and morphology. Four of the species belong to the family Allocreadiidae, 1 to the Clinostomatidae, 2 to the Lecithodendriidae, 1 to the Cephalogonimidae, 2 to the Gorgoderidae, 6 to the Plagiorchiidae and 18 to the Reniferidae. For each species the chromosome number was found to be constant, although variations occur within families and genera. Chromosome numbers of 7, 8, 9, 10, 11 and 14 are reported for species in the various families.

Evidence, based on cytological characters, does not

support Faust and Dollfus in placing the Allocreadiidae in the same superfamily with the Plagiorchiidae but it strengthens McMullen's suggestion that the family Lecithodendriidae shows relationship to the families Plagiorchiidae and Reniferidae and should be included in the same superfamily with them.

The progressive increase in chromosome number suggests aneuploidy and it is possible that this has been a major factor in the evolution of the digenetic trematodes and other hermaphroditic animals.

LITERATURE CITED

Anderson, M. G.
1935. *Trans. Am. Micros. Soc.*, 54: 271–297.

Brooks, F. G.
1930. *Am. Jour. Hyg.*, 12: 299–340.

Byrd, E. E.
1935. *Trans. Am. Micros. Soc.*, 54: 196–225.

Cable, R. M.
1931. *Quart. Jour. Micros. Sci.*, 74: 564–589.

Cary, L. R.
1909. *Zool. Jahrb.*, 28: 575–659.

Chen, Pin-dji
1937. *Trans. Am. Micros. Soc.*, 56: 208–236.

Cort, W. W.
1917a. *Jour. Parasitol.*, 1: 65–85.
1917b. *Jour. Parasitol.* 4: 49–57.

Darlington, C. D.
1937. "Recent Advances in Cytology." 2nd ed. The Blakiston Co. Phila. 671 pp.

Dingler, M.
1910. *Arch. f. Zellf.*, 4: 672–712.

Dollfus, R. P.
1930. *Ann. Parasitol.*, 196–203.

Faust, E. C.
1919. *Biol. Bull.*, 36: 315–321.
1929. "Human Helminthology," Ch. IX. "The Trematodes or Flukes. Classification." Lea and Febiger. Phila.
1932. *Quart. Rev. Biol.*, 7: 458–468.
1939. "Human Helminthology, Ch. IX. "The Trematodes or Flukes. Classification." Lea and Febiger. Phila.

Gille, K.
1914. Untersuchungen uber die Eireifung, Befruchtung, und Zellteilung von *Gyrodactylus elegans* v. Nordmann. *Arch. f. Zellf.*, 12: 415–456.

Goldschmidt, R.
1943. *Trans. Am. Micros. Soc.*, 62: 27–56.
1902. *Zeit. Wiss. Zool.*, 397–444.
1905. *Zool. Jahrb. Abt. f. Anat.*, 21: 604–654.
1908. *Arch. f. Zellf.*, 1: 232–244.
Gregoire, V.
1909. *La Cellule*, 25: 85–99.
Henneguy, L. F.
1909. Paris: *C. R. Acad. Sci.*, 134.
Hughes, R. C., *et al.*
1942. *Am. Mid. Nat.*, 27: 109–134.
Husted, L. and Burch, P.
1946. Am. Nat., 80: 410–429.
Jones, A. W.
1945. *Jour. Parasitol.*, 31: 213–235.
Jones, A. W., *et al.*
1945. *Jour. Morph.*, 77: 285–297.
Kemnitz, G. A. von
1913. *Arch. f. Zellf.*, 10: 470–506.
La Rue, G. R.
1926. *Trans. Am. Micros. Soc.*, 45: 265–281.
McMullen, D. B.
1935b. *Jour. Parasitol.*, 21: 369–380.
1937. *Ibid.*, 23: 244–258.
Markell, E. K.
1943.
Muller, H. J.
1925. Am. Nat., 59: 346.
Pennypacker, M. I.
1936. *Arch. Biol.*, 47: 309–317.
1940. *Jour. Morph.*, 66: 481–495.
Reese, G.
1939. *Parasitol.*, 31:417–433.
Schellenberg, A.
1911. *Arch. f. Zellf.*, 6: 443–484.
Schreiner, A. and K. E.
1908. *Videnskabs. Selskabets Schrifter.* I. *Math-Naturw.*, 8: 1–28.
Schubmann, W.
1905. *Zool. Jahrb. Abt. f. Anat.*, 21: 571–606.
Talbot, S. B.
1933. *Parasitol.*, 25: 518–545.
1934. *Trans. Amer. Micros. Soc.*, 53: 40–56.
Wassermann
1913. *Arch. f. Mik. Anat.*, 83: 1–140.
White, M. J. D.
1940. *Nature* (London), 146: 132–133.
Woodhead, A. E.
1931. *Trans. Amer. Micros. Soc.*, 50: 169–188.

IS THE STING RAY'S STING POISONOUS TO VERTEBRATES OTHER THAN MEN AND FISHES?

DR. E. W. GUDGER
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INTRODUCTION

IN an extensive historical résumé (Gudger, 1944) tracing the development of our knowledge, I have answered the question in the affirmative for man. Indeed, as early as 1842, Richard Schomburgk, exploring in British Guiana, on the same day had two of his helpers stung by river rays. He wrote a careful clinical account of their cases and stated that their disorders were directly comparable to those in men bitten by poisonous snakes. Again, in 1881, a French physician, Dr. J. Crevaux, while descending the Orinoco, had a companion die from a sting-ray's sting. Had the careful observations of these two men been published separately under proper titles, the question would have been settled for man 102 years ago and corroborated 63 years ago.

The ray that stung Schomburgk's men and that caused the death of Crevaux's companion is portrayed in Fig. 1. The generic name, *Potamotrygon* (river ray), has been given to freshwater rays of various species in South American rivers. Here may be seen well out toward the end of the tail the sting or stings (they are often multiple) which J. Vellard used in his experiments on lower vertebrates. These experiments will be described further on. But since no stings of South American river rays are at hand for purposes of separate illustration, the spines of two common marine rays of our Atlantic coast will be portrayed in Fig. 2.

DISCOVERY OF THE POISON GLANDS OF THE STING

Our definite knowledge of the poisonous sting dates from 1916 to 1924 inclusive. During these years, Dr.

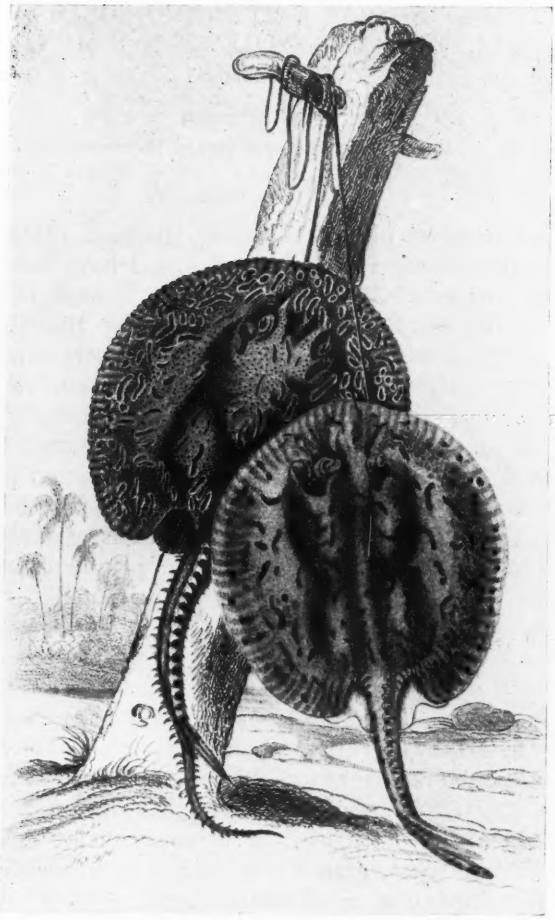

FIG. 1. *Potamotrygon histrix*, the Freshwater Sting Ray of South America. Note that one ray has one sting, the other two. It was probably a ray of this species that wounded Richard Schomburgk's Indian companions and that brought about the death of Dr. Crevaux's attendant. (After Robert H. Schomburgk, 1843.)

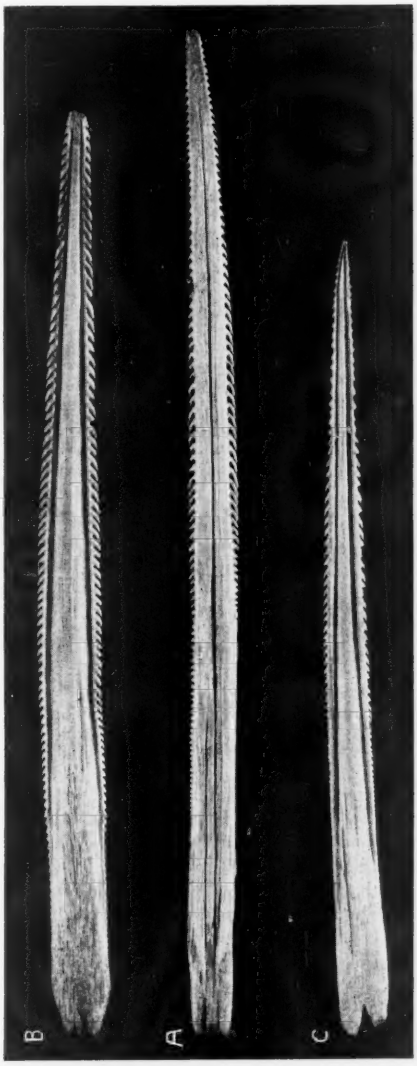

FIG. 2. Spines of Two Kinds of Stings of Sting Rays from Beaufort, N. C. A, dorsal; and B, ventral surfaces of spines of *Dasyatis say:* C, ventral aspect of bi-pronged spine of *Actobatus narinari.* On the ventral surface of each spine, note the median ridge and, on either side between this and the teeth, a marked lateral groove in which the poison gland is located. (After Gudger, 1944.)

H. M. Evans clearly demonstrated for the first time the presence of glandular poison-secreting masses at the base of and extending backward on each side of the central ridge on the ventral surface of the spine of a sting ray. However, he treated no case of stinging by a ray, nor did he do any experiments on the effects of the sting-gland product on any of the lower vertebrates.

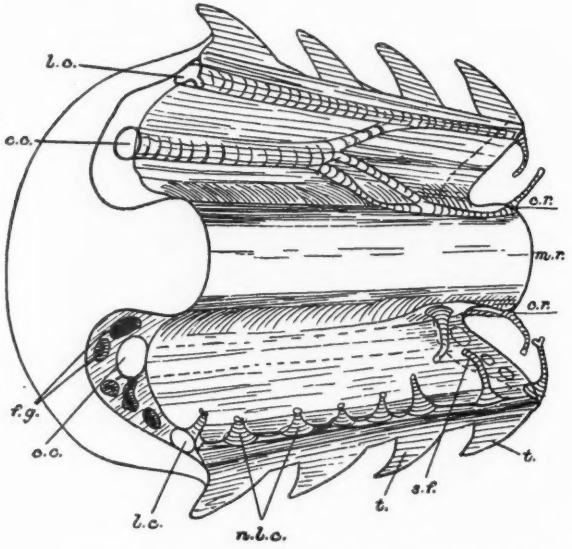

FIG. 3. Diagrammatic Drawing of a Portion of Spine and Poison Gland of *Trygon pastinaca*, the European Sting Ray, showing schematically the arrangement of canals and relations of nipples, filaments and denticles. *cc.*, central canal; *c.r.*, canal of ridge; *f.g.*, follicles of gland; *l.c.*, lateral canal; *m.r.*, median ridge; *n.l.c.*, nipples of lateral canals; *s.f.*, secreting filaments; *t.*, tooth pointing backward. (After Evans, fig. 7, 1916.)

Evans gives an excellent diagrammatic figure showing the underside of a spine with its poison glands on either side of the central ventral ridge of the spine. This drawing is reproduced as Fig. 3. The poison, secreted by the glandular tissues, is collected in the central and lateral canals and carried to the nipples and secreting filaments of the lateral canals. When the spine is driven into a

man's leg or an animal's body, pressure is exerted on the mass of secreting tissue, on the canals and on the nipples. Whereupon the poison is forced out into the tissues lacerated by the teeth of the spine and the stung animal is very quickly poisoned.

Experiments with the Poisonous Sting

Since it is known that man is stung, poisoned and sometimes (fortunately infrequently) killed by sting rays, it would be of interest to know if man's nearest relatives, the anthropoids, would be affected by the sting. No experiments with the stings have ever been done on them or on the ordinary monkeys so far as this search goes. However, there is every reason to believe that such stingings would be followed by poisonings. Excepting sharks and one reptile, no record has been found of any vertebrate lower than man ever having been stung in nature by a sting ray.

However, extensive experiments with the sting on various vertebrates other than man and the fishes were carried on by J. Vellard on the Araguaya River in central Brazil in 1931–1932. The poisonous rays of the Araguaya are akin to those of the Orinoco and other rivers of Guiana. Vellard had three men patients who had been stung and, as his careful report shows, badly poisoned. Furthermore, he performed various experiments on mammals, birds, reptiles and amphibians, using fresh stings, dried stings and various solutions of the venom. However, they are so interesting and important that they will now be described very carefully for each group of vertebrates other than fishes.

Vellard is the first and, so far as I know, the only man to make such experiments and to publish the results. His reports are badly jumbled and hard to follow. When he uses fresh stings as such or solutions of the venom from them in sterile water, he is clear. But when he uses dried stings or dried venom in various solutions and then estimates their strengths in fractions of that of a fresh sting

it is difficult to follow him. Below I present those experiments that most nearly reproduce the stinging and poisoning of man by the ray in active life.

Action of the Venom on Various Mammals

Vellard carried on experiments on dogs, mice, a rabbit and a guinea pig.

Dog No. 1, weighing 8.5 kg., had a fresh sting driven deep into the large muscle of the left thigh and left in the wound for two minutes. The reactions of the dog were quite similar to those of men stung by rays. There was great pain, evidenced by cries, marked swelling, trembling, abrupt movements, torpor and immobilization of the limb; after 8 days the dog recovered with some atrophy of the muscles. The explanation is not clear. Perhaps the sting had been used by the ray just before capture. Or perhaps the sting was driven into muscle and did not penetrate a blood vessel or a nerve. Or perhaps this good-sized dog was strongly resistant to the venom. Few men struck by rays die even when badly poisoned.

Dog No. 2, weighing 7 kg., received in the muscles of the left thigh 2.5 cc of a solution of venom in sterile water made from and equal in strength to a fresh spine. The animal reacted with the usual clinical symptoms. But presently it grew quieter, the swelling decreased, and at the end of 2 days the dog was nearly normal.

Dog No. 3, also weighing 7 kg., had a dried spine driven deeply into the muscles of the left thigh. There was some slight swelling, but this disappeared at the end of 36 hours. From this it is evident that the venom loses strength in drying. Hence the Indians' arrows when tipped with dried sting-ray spines are not particularly dangerous; and the longer the time of drying, the less the virulence.

Three mice were also experimented on as follows: No. 1 (30 gr.) was pricked under the skin of the back with a fresh spine. The normal symptoms followed, and the ani-

mal died in 70 minutes. No. 2 (31 gr.) had an injection in the thigh of 0.5 cc of a solution in boiled water of venom from a fresh spine, and died in 44 minutes. Mouse No. 3 (32 gr.) had an injection of 2.5 cc of a solution of venom from a fresh spine in sterile water. Death followed in 11 minutes.

Apparently mice are more susceptible to sting-ray poison than are dogs. However, their small size must undoubtedly have much to do with it.

A rabbit and a guinea pig were each injected with a solution of the dried venom. Both were poisoned, but both recovered. It does not seem necessary to give the details, particularly since the estimates of the strength of the dosages in terms of a fresh spine seem rather farfetched. One solution was in normal saline, the other in "eau de chaux" (lime water ?).

Experiments on Birds Inconclusive

Two pigeons were injected with a solution (in "eau de chaux") of dried venom. These experiments come under his heading "Injections Endoveineuses," but the particular veins are not indicated. Both pigeons exhibited symptoms of poisoning but both quickly recovered. One wonders as to the virulence of venom in "eau de chaux."

For the sake of completeness of experiments in the vertebrate series, it is very unfortunate that Vellard did not use fresh spines or a solution of fresh venom in boiled water, and that he did not use other birds also. Had he done so, it may be inferred that the pigeons would have at least been badly poisoned.

The Venom Fatal to Reptiles

A Turtle.—For this there is fortunately a report of an attack in nature on this animal by a ray. It is the only recorded account of a ray stinging an animal other than a man or a shark. The record was made by Lo Bianco in 1899.

There were in the same tank in the aquarium of the

Zoological Station at Naples three sea turtles and four Mediterranean sting rays (Trygons). One of the rays died, and examination showed that an entire spine had been torn off its tail. A few days later one of the turtles refused to eat and presently died. Examination revealed the lost spine buried deeply in the flesh under the turtle's right forward flipper, piercing skin and muscles and making a wound 3 to 4 cm in length and breadth. An interesting fact is that the tissues surrounding the sting were of a violet color, such as has been noted in areas around a sting-ray wound in man.

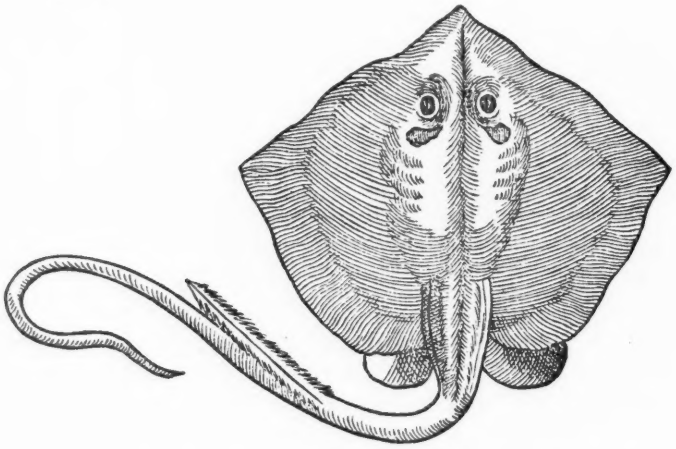

FIG. 4. "*Pastinaca marina,*" the European Sting Ray. In the figure of this ray (the first ever published in a scientific book) the spine has been drawn disproportionately large. (After Belon, 1553.)

The only figure of a Mediterranean sting ray available is a reproduction of the first illustration of this ray ever published—Belon's *Pastinaca marina,* 1553 (Fig. 4). It is called Trygon because of the three angles made by the head and the pectoral fins. Other than the spine, which is drawn disproportionately large, it fairly well portrays a Mediterranean Trygon of to-day.

A Lizard.—Vellard injected into a specimen (20 cm long) 0.5 cc of a solution in boiled water of venom pre-

pared from a fresh spine. The dose corresponded to one fourth the strength of a fresh spine. The lizard reacted immediately and died in 41 minutes.

The Venom Poisonous to Amphibians

Toads and frogs being readily obtainable, Vellard experimented on four of each kind.

Toad No. 1.—Into the back of a toad weighing 25 gms. a fresh spine was driven and at once retracted. Then followed the reactions of poisoning followed by death in 125 minutes. Toad No. 2, weighing 20 gms., was wounded in the same way as No. 1 and with the *same spine*. Then followed the same but lighter symptoms. At the end of 48 hours the swelling had disappeared and there was no necrosis of the tissues. Evidently most of the poison had been absorbed by the first toad. Toad No. 3, weighing 30 gms., was injected in the muscles of the thigh with 1 cc of a solution of fresh venom in sterile water equal in strength to one fourth a fresh spine. It was at once strongly affected and died in 14 minutes. Toad No. 4, of 40 gms. weight, received in the dorsal lymph sac 2 cc of a sterile-water solution equal in strength to one fresh spine. The usual symptoms were followed by insensibility and death in 95 minutes.

Four frogs were also experimented on with results as follows:

Frog No. 1, weighing 70 gms., was pricked with the same spine used on toads 1 and 2. The spine was driven into the muscles of the right thigh and left in place for one minute. Reactions less violent than those in the toads came at once, and at the end of 48 hours the frog was entirely normal. Evidently almost all the venom had already been absorbed by the toads. Frog No. 2, a female of 45 gms. weight, had an injection of 1 cc of a solution from a fresh spine in sterile water, equal to the strength of one half a fresh spine. The animal became very ill but recovered at the end of 12 hours.

Two frogs were injected in the dorsal lymph vessel with

like results: No. 3, weighing only 7 gms., received 0.3 cc of a sterile-water solution of a fresh sting, equalling one seventh the strength of a fresh spine. The symptoms of acute poisoning were quick, and it died in 5 minutes. The dose was very small but effective. Frog No. 4, another specimen of only 6 gms. weight, was also injected with 1 cc of solution equal in strength to half a fresh spine. The reactions were immediate and progressive to death in 11 minutes.

Vellard offers no explanation for the different results from the reception of the same dosage in frogs 2 and 4. Possibly the matter of size is the deciding factor. Nor does he comment on the fatalities in the two frogs of like weight injected in their dorsal lymph sacs.

From the preceding it is clear that the venom of the sting ray's sting is poisonous to members of all the great groups of vertebrates from man down to and including the Amphibia. Furthermore, there is every reason to conclude that were the dosage of fresh venom sufficiently large, death would follow in many if not all cases.

I have shown recently (Gudger, 1946) that there is no evidence available that sting rays ever strike bony fishes or other rays. However, it is quite different for sharks. There is abundant evidence that four kinds of sharks feed on sting rays and in the process are often stung. Stings have been found embedded in the jaws, in the external mouth parts, in the throat regions and in other parts of the outer body. Furthermore, numerous free stings have been found in the stomachs of several sharks.

The sharks, which feed on sting rays and are found with spines, are the hammerhead (*Sphyrna zygaena*), the tiger shark (*Galeocerdo tigrinus*), the black-tipped (*Carcharhinus limbatus*) and the sharp-nosed shark (*Scoliodon terrae-novae*). Further observations will probably extend this list.

LITERATURE CITED

Gudger, E. W.
1944. *Bull. Hist. Med.*, 1944, Vol. 14, 467–504, 12 figs.
1946. *Scientific Monthly*, Vol. 63, 110–116, 4 figs.
Lo Bianco, Salvatore
1899. *Mitth. Zool. Sta. Neapel*, 1899, Vol. 13, p. 549.
Vellard, J.
1932. *C. R. Acad. Sci.*, 1931, Paris, Vol. 192, 1279–1281.
1932. *Mem. Soc. Zool. France*, 1932, Vol. 29, 513–539.

REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Human Destiny. By LECOMTE DU NOÜY. New York: Longmans, Green and Co., 1947: i–xxi, 1–289. $3.50.

OF all the fields of science, biophysics is the branch which attempts to explain the phenomena of life by the mechanistic laws which apply to inanimate objects. These attempts have always proved to be inadequate and are recognized as being so by the distinguished biophysicist who is the author of this book. Moreover, his studies have convinced him that "the laws which govern the behavior of substances in the inorganic world seem to be quite inapplicable when the same chemical elements are combined in the living organism." In fact, "the laws of inorganic evolution contradict those of the evolution of life."

The object of the book is to demonstrate by arguments based on scientific methods and on mathematical reasoning "that they both lead to the necessity of admitting the intervention of a transcendent, extra-earthly force in order to explain Life." The history of the earth as well as that of the living world illustrates this universal guiding principle in all aspects of evolution, both inorganic and organic. Such evolution has led by devious paths but unerringly to nature's ultimate goal, which is the human soul. The directing force has been called by many names, the most appropriate of which is God.

The possibility that chance alone may have led to such a combination of chemical elements as may have resulted

in the origin of life is shown to be so remote as to be mathematically negligible. But having been once created there arose the possibility of emergent evolution, leading to organisms of ever increasing complexity and of that infinite number of varieties long since perished or now in existence, as well as those that are to be.

Man's evolution seems to have already reached its culmination in so far as his anatomical, physiological and intellectual development is concerned, since no progress in those directions has been discernible during the past several thousand years. But the goal which was evidently set by the Creator will not have been reached until man has attained perfection in his moral and spiritual development. His further evolution toward this goal must be guided by his own efforts, since he has now acquired a knowledge of good and evil, with freedom of choice, and a conscience. By example and precept he can transmit to future generations the moral and spiritual values which a few have already attained. This is what the author means by "telefinalism." The philosophical implications of these ideas are obviously of the utmost importance to human welfare.—WESLEY R. COE, *Yale University, New Haven, Connecticut.*

Forest Soils and Forest Growth. By S. A. WILDE. Waltham, Mass.: The Chronica Botanica Co. (New York: G. E. Stechert and Co., agents), 1946: i–xx, 1–241, figs. 1–38, pls. 1–7. $5.00.

PROFESSOR WILDE has produced a well-organized, well-written book that should be of interest not only to foresters, but also to soil scientists, ecologists, and those persons directly concerned with the production phases of the lumbering industry. The chapter titles indicate a broad, inclusive treatment of the subject matter. After an historical introductory chapter, there are a group of chapters dealing with the genesis of forest soils, genetic soil groups of the world, and the forest cover. Then follow chapters on the physical properties of forest soils, the chemical properties of forest soils, the organisms of forest soils, forest humus, soil-forest types and forest soil

survey. The next four chapters are devoted to a discussion of reforestation, amelioration, productivity, and management of forest soils. The final five chapters are concerned with the various cultural practices necessary in the establishment and management of forest nurseries.

There are over 600 literature references, both a subject and author index, and seven excellent plates. The illustrations are exceedingly well done, and there are some cleverly executed vignettes at the end of many of the chapters. A few minor typographical errors occur, while Plate 6 (*Center*) seems to be incompletely labeled.

The author and publisher deserve to be congratulated on the neat, attractive format, and the excellent printing and binding of the book. However, it is regrettable that the paper shortage forced the publisher to economize by printing portions of the index on the reverse side of some of the plates.

American soil scientists will have reason to be grateful to Wilde for making available to them the extensive European literature of forest soils, most of it written in German and Russian.—Thomas W. Whitaker, *U. S. Department of Agriculture, Box 150, La Jolla, California.*

Fleas of Western North America. Their relation to the public health. By Clarence Andresen Hubbard. Ames, Iowa: The Iowa State College Press, 1947: i–viii, 1–583, many figures and 5 unnumbered plates. $6.00.

This is a thoroughly excellent addition to entomological literature. It would have been useful had it been merely a systematic review of the fleas of the area included, but it is far more than this. There is a chapter of biographies of students of fleas who have built up our knowledge of the fleas of North America. There are chapters on the medical importance of the fleas, on the technique of field collecting and the preparation of material for study and on the taxonomic anatomy of the group. The systematic review contains keys to the families, genera and species with the pertinent descriptive material under each category. For each species

there are illustrations showing the important diagnostic details and a list of hosts with collecting records.

In addition to this purely entomological material there is a discussion of the hosts, their distribution and habits as related to the occurrence of fleas. All records are included of fleas that have been taken from the individual host species. In discussing the natural history of the hosts the author presents a very considerable amount of material gained by himself in extensive field collecting. There is an extended bibliography. The series of indices includes an index of flea species, an index of authors and the species described by them, and a general index.

It would be difficult to suggest anything else that the author could have done to increase the usefulness of the book. The only criticism that the reviewer—a rather notoriously critical individual—can make has to do with the illustrations, and this criticism applies to most entomological books. Following the general requirements of editors, the illustrations are too crowded and too much reduced to be of the greatest usefulness. Some are so crowded that it is difficult for the eye to discriminate among the various parts and are so much reduced that it is difficult to read the legends which, very commendably, have been inscribed on the illustrations themselves. But it will probably be a long time before editors, confronted with the little matter of cost, will be willing to subscribe to the view that if there must be sacrifice and condensation it should not be at the expense of the illustrations.—G. F. Ferris, *Leland Stanford Junior University, Stanford University, California.*

NOTICES OF NEW BOOKS

Insect Microbiology. By Edward A. Steinhaus. Ithaca, New York: Comstock Publishing Company, Inc., 1946: i–x, 1–763, figs. 1–256, tables 1–2. $7.75.—Rarely is an investigator trained in both entomology and the compounded science of microbiology, embracing the fields of bacteriology, protozoology, mycology and allied sciences. Dr. Steinhaus is not only such an investigator, but he also has the ability to write lucidly, succinctly and enter-

tainingly as is abundantly evidenced by this "account of the microbes associated with insects and ticks with special reference to the biological relationships involved." In introducing the entomologist and the microbiologist to each other, Dr. Steinhaus brings together, for the first time, a vast wealth of information which will be of lasting interest to the student of general biology, ecology, pathology, epidemiology, public health, parasitology and physiology as well as microbiology and entomology.

There are comprehensive chapters on bacteria, yeasts, fungi, viruses, spirochaetes, rickettsia and protozoa, in which the various microbes are considered as adventitiously or mechanically associated with insects, as symbiotes of insects, a food for insects, as the etiological agents of insect diseases and as plant or animal pathogens transmitted by insects. Listed are more than 300 species of bacteria found associated with insects and ticks. In two of the longest and best chapters dealing with Intracellular and Bacteriumlike and Rickettsialike Symbiotes and the relation of Rickettsia to insects and ticks, the properties, characteristics, synonyms and biological relationships of a large number of little known rickettsial species are given, including information on several rickettsial diseases of man and other animals.

Steinhaus points out that for the most part the known relationships of fungi to insects are parasitic or semiparasitic. Fungous diseases are apparently Nature's way of controlling biologically the numbers of a given species of insects. Although man's efforts to control destructive insects by the artificial propagation of microbic diseases have not been too successful, further investigations along this line hold much promise for the future. Most fascinating are the discussions of fungous gardens of ants and ambrosia fungi in beetle galleries.

Protozoa have been found associated with nearly all orders of insects. In one reported investigation 34 per cent of the species and 19 per cent of the individual insects harbored protozoa. Many of the latter, such as species of *Trypanosoma, Leishmania, Plasmodium* and *Babesia*, cause infectious diseases in man and other animals. Approximately 250 species of flagellates are listed in the chapter entitled Protozoa and Termites, in which the social life, natural history, physiology and symbiotes of termites are discussed.

The chapter on Immunity in Insects is more descriptive of the problems than informative, which is quite understandable, for,

as Dr. Steinhaus points out, studies in this almost virgin field have had only a laggard start. The unique training of the author is reflected in the chapter on Methods and Procedure, in which specialized techniques are described for studying insect microbiology while sparing the reader detail readily found in cited reference books. A bibliography of 1,744 references complete with titles and inclusive paging together with an author index and a subject index add to the value of the book for research and teaching purposes.—CLAUDE E. ZOBELL, *Scripps Institution of Oceanography, University of California, La Jolla, California.*

Wyoming Fishes. By JAMES R. SIMON. (Cheyenne, Wyo.), 4: 1–129, frontisp., figs. 1–92, 11 vignettes. $1.00.—Accurate and informative, this manual provides means for the ready identification of the native and introduced fishes of Wyoming. It also presents data on habitats and on reproductive and feeding habits. The author states that "it is not intended for the advanced ichthyologist, but rather for the student and fisherman and all other conservation-minded individuals." It will, however, be much used by fish scientists, for it is a valuable contribution to ichthyology, as well as a model popular report on the fishes of a state.

History of the North American Bird Fauna. By ERNST MAYR. Wilson Bulletin, 58, 1946: 3–41, col. frontisp., figs. 1–4. The North American bird fauna is here subjected to a kinetic analysis in terms of the inferred origin of its elements. The inferences are based largely on the circumstantial evidence of present-day distribution, for the fossil record is too incomplete to be trustworthy. Most of the conclusions are stated with reserve, though such words as "prove," "undoubtedly," and "must" are used oftener than I would. The North American bird fauna is regarded as too distinctive and too mixed in origin to be treated either as the Nearctic division of the Holarctic realm or as a part of the Neotropical. It is pointed out that both during and after the existence of the Middle American straits, the South American bird elements moved into North America much more freely than the reptiles or mammals did. Fresh-water fishes showed even less incursion. For such groups the recognition of an Holarctic region or fauna is more strongly indicated.

Contributions from the Laboratory of Vertebrate Biology, University of Michigan.—Numbers 31 to 38 of these Contributions, recently distributed, markedly advance our knowledge of heredity and speciation in several groups of organisms.

In Contribution No. 31 (1946: 1–24), on "Speciation in Limpets of the Genus *Acmaea*," Avery Ransome (Grant) Test concludes "that in the genus *Acmaea*, in addition to the process of speciation based on geographical isolation, there has been, and perhaps even more frequently, speciation from eurytopic ancestors by the process of ecological isolation and selection." This process derives its importance in this group from two circumstances—great differences in the ecological conditions of several microhabitats within the intertidal belt and the extremely small effective range of the individual.

In Contribution No. 32 (1947: 1–24), "Occurrence of Parallel Series of Associated Physiological and Morphological Characters in Diverse Groups of Mosquitoes and Other Insects," William Hovanitz shows that several characters are associated in parallel fashion in the geographical variation in various groups of insects and that the differences are attributable to genetic changes in the speed of body metabolism. In 1926 I reached a like conclusion in regard to variation in fishes.

In No. 33 (1947: 1–21, fig. 1), C. W. Cotterman presents, with a plethora of mathematics, "A Weighting System for the Estimation of Gene Frequencies from Family Records."

In No. 34 (1947: 1–20), Lee R. Dice publishes the results of experiments, reported at the last meeting of the Society for the Study of Evolution, demonstrating the "Effectiveness of Selection by Owls of Deer-mice (*Peromyscus maniculatus*) Which Contrast in Color with Their Background."

In No. 35–38, (1947), W. Frank Blair analyzes the genetics, variation, gene-frequencies and selectional significance of pelage color in wild mice of the genus *Peromyscus*.

The New Journal, "Heredity"

Oliver and Boyd, publishers of London and Edinburgh, have announced the founding of a new journal entitled *Heredity* under the editorship of Cyril D. Darlington and Ronald A. Fisher and with the collaboration of G. W. Beadle, T. Caspersson, Th. Dobzhansky, B. Ephrussi and O. Winge. The journal will be issued three times per year and each number will consist of about

128 pages. The subscription price is £2 or $8.00 per year. Orders should be placed with the publishers, Messrs. Oliver and Boyd Ltd., Tweeddale Court, High Street, Edinburgh.

This journal was established because the editors and publishers feel that "Genetics has to be introduced to a wider audience, indeed to the whole world of Biology since the whole world of Biology needs it. For this purpose more general introductions, more general statements and more general views are needed. For this purpose too, pure research and applied research are in our view closely related and mutually important. Technique and theory can each be of general value. Speculation with regard to the future and revaluation of the past must each demand our attention. Review and controversy and the teaching, methodology and administration of our science complete the list of our interests."

In answer to the question, "To whom is *Heredity* addressed?" the editors reply, "To the Botanist and Zoologist by way of evolution and systematics. To the Physiologist by way of cytology and experimental technique. To the Medical research worker on account of both diagnosis and treatment; on account, too, of both man and his enemies. To the Social Scientist since the study of nature and nurture is the foundation of his work. To the Agriculturist since Plant and Animal Breeding is the key to his future. And finally to the Physicist and the Chemist since here the bridge is being built which joins their sciences with biology."

The first number of *Heredity* appeared in July. It includes an interesting review of genetic research carried on in Britain during the war years, 1939–1945, a valuable bibliography of genetic research published in Italy and Germany during this same unhappy period, plus eight papers reporting the results of genetic research. Contributors to Volume I include well-known geneticists and cytologists from Britain, Denmark, Italy, Switzerland, Sweden, Peru and the U.S.A. *Heredity* is planned as an *International* journal.—G. W. Beadle, California Institute of Technology.

SHORTER ARTICLES AND DISCUSSION

CHROMATIN ELIMINATION AND THE CILIATE MACRONUCLEUS

CHROMATIN elimination is known to occur in a number of animals and is apparently of different kinds. First, there is the loss of parts of chromosomes in *Ascaris megalocephala* where, in the early cleavages of the egg, the blastomeres destined to give rise to the somatic tissues lose the ends of their chromosomes and these ends are absorbed by the cytoplasm (Boveri, 1887; Walton, 1924). Then, the elimination of whole chromosomes or sets of chromosomes takes place in *Sciara* (Metz, 1933) and certain Cecidomyidae (Kraczkiewicz, 1935; Reitberger, 1934, 1940; Metcalfe, 1935). Thirdly, there is the elimination of bodies which are not recognizable as chromosomes but in which there is certainly nucleic acid though not a well-defined protein framework, as in the Lepidoptera (Seiler, 1914, 1923; Kawaguchi, 1928; Fogg, 1930), Trichoptera (Klingstedt, 1931) and in *Pediculopsis* (Cooper, 1939). The third case differs from the first two in the fact that, while in the latter the whole chromosomes or ends of chromosomes have a recognizable protein framework, the former appear to be in the nature of amorphous masses of ribose nucleic acid in which a protein framework has not been demonstrated. To these three should be added a fourth case of chromatin elimination occurring in certain ciliate Protozoa where Painter (1945) has reported the formation of buds on the macronucleus of *Tetrahymena galeii* which are pinched off into the cytoplasm where they undergo absorption. Similar bud formation has been reported by Diller (1936) in *Paramecium* and by the author (1946) in *Epistylis*, where, however, this has been regarded as part of a reorganization process of the macronucleus. The real nature of these buds is not clearly understood and it is still a matter of doubt whether they are associated with protein or not. But the recent finding by Painter (1945) of the formation of the chromosomes in the macronucleus of *Tetrahymena galeii* leads one to believe that the macronuclear buds formed in this ciliate are probably associated with protein material, though whether these buds represent whole chromosomes or parts of the chromosomes still remains to be determined.

The real difficulty of considering the loss of such valuable material as chromatin without the complete upset of genic equipment of the organism was overcome by the suggestion of Muller

and Painter (1932) that the material so discarded was inert heterochromatin, whose loss, while having some general effects on the organism, did not produce any significant specific effects. On this basis especially, has been explained the loss of the "limited chromosomes" of *Sciara* as well as the "B" chromosomes in maize roots.

But the significance of the loss from the nucleus of this heterochromatin would itself have to be explained, and both in the Metazoa where such loss has been reported and in the Protozoa where the macronuclear buds are thrown into and absorbed by the cytoplasm, Painter (1945) sees a process of distribution of nucleic acid in the cytoplasm, where it initiates phenomena of protein synthesis, rapid growth or elaboration of secretory products. In fact, the actual process of the passage of nucleic acid from the nucleus into the cytoplasm of the toad's egg during oogenesis has been described by Painter, who believes that the reason for the discharge of so much nucleic acid in the egg cytoplasm is the formation of new chromosomes during cleavage and protein synthesis connected with the growth of the cytoplasm. In the Protozoa, Painter's (1945) studies have shown large accumulations of ribose nucleotides in the cytoplasm of many ciliates and it would be a natural assumption that these cytoplasmic nucleotides must come from absorbed macronuclear buds.

My observations on *Epistylis* (Peritricha: Epistylidae) lead me to the same conclusion. Here too the macronucleus gives off buds or a total fragmentation of the macronucleus takes place. It is probable that the process of hemixis described by Diller (1936) in *Paramecium* and confirmed for *Epistylis* by the author (1946) marks the first step of such a macronuclear change. Neither Diller nor the author has been able actually to watch the disorganization and absorption of the macronuclear fragments, but it is more than likely that these nuclear fragments discharge their nucleic acid content into the cytoplasm for some later use.

I am, however, concerned with another phenomenon. It is a matter of common knowledge that prior to conjugation, the macronucleus breaks up and is distributed in the cytoplasm where it undergoes absorption and disappears. While the words "absorption" and "disappearance" were satisfying enough to the early workers on Protozoan life-histories, they are hardly so to the modern cytologist who would like to know what has ultimately happened to this valuable and dynamic material in the cytoplasm.

I suggest that the process of breaking up and absorption of the macronucleus in the cytoplasm of the conjugating ciliate is nothing but the same essential process of dispersal of its nucleic acid content in the cytoplasm. It is merely a phenomenon of chromatin elimination. The role of such nucleic acid has already been discussed. If the role is rapid growth or production of secretory products in the Metazoa, there is probably a complete reorganization of the cytoplasm in the Protozoa. The particular need for the nucleic acid in the cytoplasm at the time of conjugation is probably to be found in the thorough overhauling to which the cytoplasm is subjected.

The phenomenon as I envisage it is this: The macronucleus in each conjugating individual discharges all its nucleic acid into the cytoplasm. The micronucleus divides a number of times, giving rise to a number of daughter nuclei. The absorption of all but one of these products follows, while the development of the stationary and migratory nuclei occurs from the surviving daughter. After the completion of conjugation and at the end of the preliminary divisions of the synkaryon attended by divisions of the body, ultimately two nuclei are left in each individual. While one remains small, the other enlarges and becomes the macronucleus. The small one is the micronucleus.

The difficulty met with in the explanation of the process of disappearance and absorption of the macronucleus also occurs when one considers the "enlargement" of one of the nuclei of the exconjugant into the macronucleus. Nuclear material can not enlarge nor can it add to its volume without important changes occurring in the organism. The nucleic acid content of a given nucleus is a specific entity and can not be disturbed without consequence. "Enlargement" must mean, and to my mind only means, the reacquisition, from the cytoplasm, of the nucleic acid which had been earlier distributed through it at the time of the disorganization of the macronucleus prior to conjugation.

That, to my mind, is the explanation of the picture. But in this explanation are hidden a number of other important questions which require answering. Why should only one of the nuclei enlarge and thus acquire the nucleic acid from the cytoplasm? Since the two nuclei are in the nature of sisters; what is the particular factor that determines the enlargement of one and not the other, or both? Is the nucleic acid being reabsorbed the same as that which was liberated into the cytoplasm? These

are vital questions and can be answered only after a thorough reinquiry into the constitution, behavior and function of the two nuclei.

If this explanation is correct, the old and classical ideas regarding the reformation and reorganization of the macronucleus must undergo considerable change. That the micronucleus after conjugation is different from that which existed before, will be granted. Syngamy has taken place. But that the macronucleus of the exconjugant is different from that of the original macronucleus can not be wholly agreed to. It must essentially be the same nucleic acid, but against a new background—a new micronucleus and its descendant. Associated with a new basic framework derived from syngamy, the enlargement is merely the restitution of the original nucleic acid.

One question still remains to be answered, whether the macronucleus and its buds are in the nature of heterochromatin. On *a priori* grounds it would appear so. The earlier observations that the macronucleus did not form the chromosomes appear to lend support to this view. In fact, the total fragmentation of the macronucleus in *Paramecium* (Diller, 1936) and *Epistylis* (Seshachar, 1946) would lead one to believe that here is material that could be lost into the cytoplasm without upsetting the genic balance of the organism.

Staining reactions prove this. The macronucleus of *Epistylis* gives a brilliant positive reaction with Feulgen, while the micronucleus at least in some species of the genus (Seshachar, in press) shows an almost complete negative reaction. This difference in the staining reactions of the two nuclei is significant and indicates a possible difference in the nature of the chromatin in the two nuclei. It is possible that the macronucleus is made up wholly of heterochromatin. However, other tests would have to be applied before this can be affirmed.

Recent work by Sonneborn (1941) on *Paramecium* has brought to light the conspicuous part which the macronucleus plays in the determination of the genetic behavior of this ciliate and it is all the more important that a correct understanding of the structure and behavior of the nuclear apparatus of ciliates be obtained for a full realization of the importance of these organisms.

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LITERATURE CITED

Boveri, Th.
1887. *Anat. Anz.*, 2: 688.
Cooper, K. W.
1939. *Chromosoma*, 1: 51.
Diller, W. F.
1936. *Jour. Morph.*, 59: 11.
Fogg, L. C.
1930. *Jour. Morph.*, 50: 413.
Kawaguchi, E.
1928. *Z. Zellforsch.* 7: 519.
Klingstedt, H.
1931. *Acta Zool. Fenn.*, 10: 1.
Kraczkiewicz, Z.
1937. *Cellule*, 46: 57.
Metcalfe, M. E.
1935. *Quart. Jour. Micros. Sci.*, 77: 585.
Metz, C. W.
1933. *Biol. Bull.*, 54: 333.
Muller, H. J. and T. S. Painter
1932. *Z. Indukt. Abstamm. u. Vererb.*, 62: 316.
Painter, T. S.
1945. *Trans. Conn. Acad. Arts and Sci.*, 36: 443.
Reitberger, A.
1940. *Chromosoma*, 1: 391.
Seiler, J.
1914. *Arch. Zellforsch.*, 13: 159.
Seshachar, B. R.
1946. *Cur. Sci.*, 15: 198.
Sonneborn, T. M.
1941. "Cytology, Genetics and Evolution." Univ. Penn. Press, Philadelphia.
Walton, A. C.
1924. *Z. Zell. u. Gewebelehre*, 1: 688.